LIFE HISTORY OF THE NORTHERN MADTOM, *NOTURUS STIGMOSUS* (SILURIFORMES: ICTALURIDAE),IN THE LICKING RIVER, KENTUCKY

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ABSTRACT

The northern madtom, *Noturus stigmosus* Taylor (Siluriformes: Ictaluridae), is a small, riffle-dwelling ictalurid catfish found in isolated populations throughout the Ohio River and upper Mississippi River drainages. Although it has a wide distribution, this fish is rarely abundant in a given area, and is thought to be declining throughout its range. Little is known about the life history and reproductive biology of *N. stigmosus*, although these data are critical for conservation of the species.

Habitat use, reproduction, demography, growth, and diet of *N. stigmosus* were studied from specimens collected in the Licking River at Moore's Ferry, Kentucky between 19 June 2001 and 12 October 2002 and specimens vouchered in the Morehead State University collection from the same site. Intra- and interspecific habitat partitioning and competition was examined from collections made in the summer 2001-2002 of *N. stigmosus*, *N. miurus*, and *N. flavus*.

Individuals of *N. stigmosus* were most common in mixed substrates consisting of fine gravel, coarse gravel, and cobble. Juvenile *N. stigmosus* occupy a narrow niche within adult microhabitat parameters. Moderate current was preferred (mean = 0.50 m/sec), although young in their first month of life were found in slightly slower water (mean = 0.48 m/sec) immediately above a riffle. Submerged aquatic vegetation had little effect on adult microhabitat selection; but the presence of juveniles was positively correlated with *Potamogeton* beds. Gonadosomatic indices suggest a discrete spawning event in late June to late July, with sexual maturity reached during the second summer of life at approximately 60 mm standard length (SL). Clutch size is estimated at 70 – 100 eggs per female based on examination of mature ovaries. A single nest, containing approximately 40 metalarvae 11-14 mm SL, was found on 7 July 2002 under a large rock in moderate current (0.49 m/sec) at the head of a riffle in 25°C water. *Noturus stigmosus* lives at least three years at Moore's Ferry, reaching 87 mm SL. Growth is rapid, with young reaching 35-40 mm SL by three months and 45-50 mm SL by one year. Population density is highly volatile, and recruitment is likely dependant on seasonal water conditions. The diet of small *N. stigmosus* was dominated by Chironomidae, Ephemerellidae, and Hydroptilidae, while larger individuals fed heavily on Simuliidae, Heptageneiidae, and Hydropsychidae. Univariate and multivariate comparisons of habitat use suggest habitat partitioning between *N. stigmosus* age classes and between the madtom species at Moore's Ferry, however little evidence for intra- and interspecific competition was found.

Noturus stigmosus exhibits life history traits comparable to other riffle-dwelling madtoms of similar size, but the spotty distribution, volatile population dynamics, and sensitivity to disturbance make these data critical for the management of this imperiled species. Dam releases from Cave Run Lake 10 km upstream significantly impact reproduction and recruitment of the Moore's Ferry population, and implications of these events for the conservation of the species are discussed.

Accepted by: David J. Eisenhour, Chair

David P. Smith

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I would like to dedicate this study to my late father, Robert L. Scheibly.

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INTRODUCTION

Literature Review

Ictalurid catfishes comprise one of the largest groups of endemic North American fishes. The madtoms (genus *Noturus*) dominate the family Ictaluridae, with 26 described species (Page and Burr, 1991), and potentially ten undescribed forms (Warren Jr., et al., 2000; Burr and Stoeckel, 1999). Madtoms are small, generally nocturnal catfishes with adnate adipose fins (Burr and Stoeckel, 1999). They are restricted to the United States east of the continental divide, although some species' ranges extend into southern Canada (Page and Burr, 1991). Sizes of these fishes range from 36 mm maximum standard length (SL) in the pygmy madtom, *Noturus stanuli* (Burr and Stoeckel, 1999), to 312 mm SL in the stonecat, *Noturus flavus* (Mayden and Walsh, 1984), but more than two-thirds of the species fall under 100 mm SL (Burr and Stoeckel, 1999). Madtoms are well-known for the venomous sting associated with the sharp spines in the leading edges of their pectoral and dorsal fins, although it is unclear whether the venom is produced by a discrete gland (Taylor, 1969) or by the cells lining the sheath of the spine (Birkhead, 1972).

Madtoms are primarily insectivores, consuming mostly chironomids, ephemeropteran larvae, and trichopteran larvae (Burr et al., 1989; Starnes and Starnes, 1985; Burr and Mayden, 1982a; and others), as well as crayfishes, other crustaceans, and rarely fishes. Feeding peaks are seen shortly after sunset (Starnes and Starnes, 1985) and just before sunrise (Burr and Stoeckel, 1999; Mayden and Burr, 1981), often following diel variations in macroinvertebrate drift (Gutowski and

Stauffer, Jr., 1993). Madtoms typically inhabit moving waters in or below riffles or in pools with cover for daytime shelter (Burr and Stoeckel, 1999). Some species burrow into the interstices of streambed gravel during the day as well (Mayden and Burr, 1981; Stegman and Minkley, 1959).

Noturus is divided into three subgenera, *Noturus, Rabida,* and *Schilbeodes*. *Schilbeodes* madtoms (10 species) are generally uniformly colored, inhabiting quiet waters with soft substrates. *Rabida* madtoms (15 species) have striking patterns of dorsal saddles that contrast with the primary body color (Etnier and Starnes, 1993). This bold coloration is cryptic against the gravel substrate (Armbruster and Page, 1996), typical of the riffle habitats members of this subgenus occupy (Etnier and Starnes, 1993). Subgenus *Noturus* consists solely of *N. flavus*, which is uniformly colored but typically inhabits swift waters with rocky substrates (Page and Burr, 1991).

Madtoms generally live three to four years, although some annual and long-lived (up to nine years) species occur. Individuals usually reach sexual maturity during their third summer, so they may contribute multiple reproductive efforts during their lifetime (Burr and Stoeckel, 1999). Growth is generally very rapid during the first summer of life, slowing as the fish ages. Growth rates approach zero during the colder months (Mayden and Burr, 1981).

Madtoms come into reproductive condition in spring. While they do not undergo the drastic changes in color and fin structure that many fishes do in the breeding season, some secondary sexual characters are seen. Males develop greatly

swollen cephalic epaxial muscles and lips, as well as swollen genital papillae. Females develop distended abdomens and swollen tissue around the genital papillae. Gonadosomatic indices (GSI, gonad weight relative to adjusted body weight) increase rapidly in spring and then taper off in summer after spawning (Burr and Stoeckel, 1999).

Madtoms nest from early May to mid-September, spawning when water temperatures reach 20°C. Nests are generally found in pools above or below riffles, at the heads of riffles, or in raceways adjacent to riffles. Most species excavate small depressions in the substrate below large flat rocks (Burr and Stoeckel, 1999), but some use human refuse opportunistically (Burr et al, 1989; Burr and Mayden, 1982a; Burr and Mayden, 1982b). Spawning behavior has never been documented in the field, but in laboratory settings, fairly elaborate courtship behaviors have been observed. Breeding pairs engage in a head-to-tail caudal embrace periodically for a period of three to four days, after which the eggs are laid and fertilized. Females generally leave the nest after spawning; males guard, clean, and fan water over the egg mass until hatching (Burr and Stoeckel, 1999).

Nest clutches consist of a single free mass of eggs that adhere to each other, and mean clutch sizes range from fewer than 25 in the speckled madtom, *Noturus leptacanthus*, to 200 or more in the checkered madtom, *Noturus flavater*, and *N. flavus* (Burr and Stoeckel, 1999). Eggs are all similar in appearance, opaque and ranging in color from cream (Burr et al., 1989) to lemon yellow (Burr and Mayden,

1982a) to amber (Mayden and Burr, 1981). Approximately one week after spawning, embryos hatch tail-first as mesolarvae. The larvae have large yolk sacs, some development of dorsal melanophores, well-developed barbels, nostrils, eyes, fins, and fin rays. Larvae are negatively phototaxic and stay on the bottom. Within one to two weeks posthatching, development progresses to the metalarval stage with absorption of the yolk sac. Beyond larval development, the ecology of young-of-the-year (YOY) madtoms is essentially unknown, but they are believed to aggregate in large schools and associate with leaf packs in streams (Burr and Stoeckel, 1999).

Interactions with other organisms are poorly known, but nest site competition has been suggested between *Noturus* species, as well as between *Noturus* and *Necturus* salamanders due to similar nest habitat requirements (Burr and Stoeckel, 1999; Burr and Mayden, 1982a). While Wildhaber et al. (1999) suggest that interspecific competition is not a limiting factor in Neosho madtom (*Noturus placidus*) populations, in certain streams competition may be expected given the similarity in habitat and diet seen in *Noturus* species. Wildhaber et al.'s position is based upon study sites with only two madtom species present, but it is common to find multiple syntopic species at a given location. For example, Starnes and Starnes (1985) report finding up to four species of *Noturus* in association with the mountain madtom, *Noturus eleutherus*, in eastern Tennessee. Competition could potentially be high; the most intense competition in fish assemblages is seen between sympatric species within a given genus (Matthews, 1998).

Predation upon adult madtoms is minimal, due to their nocturnal habits, sharp pectoral and dorsal spines, and venomous sting. However, watersnakes of the genus *Nerodia* have been observed consuming adult madtoms. Some fishes (e.g. large centrarchids, pike) eat adults as well, but generally not until other prey options have been exhausted. Nest predation has been reported from crayfishes, darters, and minnows, but this is probably negligible when guardian adults are present (Burr and Stoeckel, 1999).

Madtoms are among the least fecund of North American fishes, and over half of the 26 species have limited distributions, which makes many vulnerable to extirpation (Burr and Stoeckel, 1999). Only the Scioto madtom, *Noturus trautmani,* is considered extinct, but many are listed as threatened or endangered on the federal and state level (Warren Jr., et al., 2000; Page and Burr, 1991). Most species have some life history data available, but more are needed to uncover geographic differences. Four species have essentially no quantitative, published life history data available, including the northern madtom, *Noturus stigmosus* (Burr and Stoeckel, 1999).

The northern madtom is a deep-bodied, medium-sized species (130 mm maximum total length, TL) found in isolated populations throughout the Ohio and upper Mississippi River basins (Page and Burr, 1991; Taylor, 1969). A *Rabida* madtom and member of the *Noturus furiosus* species group (Grady and LeGrande, 1992), *N. stigmosus* is generally described as having a bold brown or black dorsal saddle pattern over a pinkish, yellowish, or tan background color (Taylor, 1969).

However, specimens from Tennessee are more boldly patterned than those from the type locality in Michigan and the rest of the upper Ohio River, and probably represent a separate species (Thomas, 2000; Burr and Stoeckel, 1999).

Some anecdotal habitat preference, nesting, diet, and sympatric species data are available for *N. stigmosus*, but quantitative data generally are lacking. Taylor (1969) described the species in his monographic revision of the genus *Noturus*, and presented some data on the ecology and life history of *N. stigmosus*. The species was found in large streams and small rivers over silt-free substrates of gravel and cobble in moderate to swift currents. Maximum length attained was 100.5 mm SL, with many specimens reaching 80 mm SL. *Noturus stigmosus* was reported to have been collected with *Noturus gyrinus, N. flavus, N. hildebrandi, N. elegans, N. trautmani, N. eleutherus,* and *N. miurus*. Taylor goes on to describe preserved material from five *N. stigmosus* nests collected in the Huron River, Michigan. These nests were all collected in mid to late July in the 1930s and 1950s. The clutches ranged in size from 89 to 141 eggs; one was taken from a nest cavity under a stone, the rest from human refuse.

MacInnis (1998) reported *N. stigmosus* nesting in artificial substrates in Lake St. Clair, Ontario, Canada. Egg masses and guardian adults were found in nests intended to attract round gobies (*Neogobius melanostomus*) in mid to late July of 1996. Species identifications, clutch size, egg diameter, and juvenile and adult lengths were all determined by video surveillance of the nests. Clutch size was estimated at 32 to 140 eggs, with spawning occurring in mid to late July. Nests

occupied by *N. stigmosus* were in sand substrates with occasional cobble and cover of *Chara*. Males were observed guarding the nests until the eggs hatched. Maximum length was measured at 129.8 mm TL, with significant dimorphism in length reported for the sexes.

Holm and Mandrak (2001) briefly commented on diet and habitat of the species in two rivers and Lake St. Clair in an update to the status of the species in Canada. Beyond these data, essentially nothing is known about the life history of *N. stigmosus*.

Rationale

Noturus stigmosus has no federal protection as threatened or endangered, but it is found sporadically throughout its range, and is disappearing on the edges of its range (Page and Burr, 1991). The fish is listed as state endangered in Ohio (Ohio Department of Natural Resources, unpubl. data), Pennsylvania (Pennsylvania Department of Conservation and Natural Resources, unpubl. data), West Virginia (West Virginia Department of Natural Resources, unpubl. data) and Michigan where it has likely been extirpated from the type locality (Carman, 2001), in need of management in Tennessee (Tennessee Wildlife Resources Agency, unpubl. data), and of special concern in Kentucky (Kentucky Department of Fish and Wildlife Resources, unpubl. data). *Noturus stigmosus* is not protected in Illinois, but it has been collected only in the Wabash and Vermilion Rivers (Illinois Endangered Species Protection Board, 1992), and is most likely extirpated from the state (Illinois Natural

History Survey, unpubl. data). The Southeastern Fishes Council Technical Advisory Committee lists *N. stigmosus* as vulnerable, meaning that it "may become endangered or threatened by relatively minor disturbances to its habitat" and "deserves careful monitoring of its distribution and abundance" (Warren, Jr., et al, 2000).

Overall, 18 of the 26 species of madtoms are listed as protected or of special concern (Johnson, 1987), mostly due to anthropogenic impacts on streams. Habitat destruction is probably the main factor in the decline of these species, but actual habitat requirements for most of them are largely unknown (Simonsen and Neves, 1992). Benthic fishes are disproportionately vulnerable to extirpations because benthic habitats are affected first by impacts on aquatic systems (Warren, Jr., et al., 2000). Impounding streams can have negative impacts on madtom populations as well. Cold hypolimnetic releases from reservoirs can potentially disrupt reproduction by keeping water temperatures below 20°C, the critical spawning temperature for madtoms (Burr, et al., 1989). Given the low fecundities of madtoms, anthropogenic impacts can have significant deleterious effects on populations, especially for species found in small, isolated populations like *Noturus stigmosus*; successive year class failures can cause large population fluctuations and localized extinctions (Simonsen and Neves, 1992).

Detailed life history data would be critical to wildlife managers to ensure the viability of *N. stigmosus* populations throughout the species' range. In addition, Burr and Stoeckel (1999) identified several areas of madtom biology where investigation is

most needed, including interspecific competition and ecology of young-of-the-year after dispersal from the nest. Finally, the life history data generated by this study will allow the examination of geographic trends in madtom biology, corroborate phylogenetic relationships within *Noturus,* and help clarify large-scale trends in North American freshwater fish biology. Thus, this study provides significant and original data on the biology of *N. stigmosus* as well as madtom biology in general.

The Licking River in eastern Kentucky provides a unique opportunity to generate these data. The river has one of the largest and most stable populations of *N. stigmosus* known (pers. comm., Brooks Burr), with at least one site (Moore's Ferry, Bath County) where the species is locally abundant.

Objectives

This study aimed to document various aspects of the biology of *N. stigmosus* to add to the life history database of madtoms. Specifically, this study examined the habitat requirements, diet, demography, reproductive cycle, nesting habits, and early development of *N. stigmosus*, along with interspecific competition and microhabitat partitioning among syntopic madtom species at Moore's Ferry.

MATERIALS AND METHODS

Site Description

The Licking River (Figure 1) flows for approximately 496 km from its origin in the Allegheny Plateau in Magoffin County to its junction with the Ohio River near Covington, Kentucky. Two main tributaries join the river along its course, the North Fork at Milford, and the South Fork at Falmouth (Burr and Warren, Jr., 1986).

Land cover consists of mesophytic forest at the river's headwaters, trending to agricultural fields and then more industrialized areas as it flows northwest (Licking River Region Team, 1998). The main stem and its tributaries are generally upland, moderate to high gradient streams with well-developed riffles and rocky substrates. Floodplains are poorly developed, but areas adjacent to the stream support numerous farms and urban areas, putting the watershed at high risk from agricultural runoff and sewage (Burr and Warren, Jr., 1986). The river has 43 dams along its course, the largest forming Cave Run Lake near Morehead (Licking River Region Team, 1998).

The site of this study was the Licking River at Moore's Ferry in Bath County, Kentucky, approximately 10 km below Cave Run Dam. Kentucky Route 211 crosses the Licking River here as a low-water ford near the town of Salt Lick. This site contains a large shoal that supports a diverse ichthyofauna (Table 1), including three species of madtoms. *Noturus stigmosus* readily is found here along with *N. flavus* and *N. miurus*. The shoal is approximately 100 - 150 m long and 30 - 35 m wide. Substrates are rocky, consisting of gravel interspersed with larger cobble and boulders. Submerged aquatic vegetation, consisting of *Potamogeton*, is very common

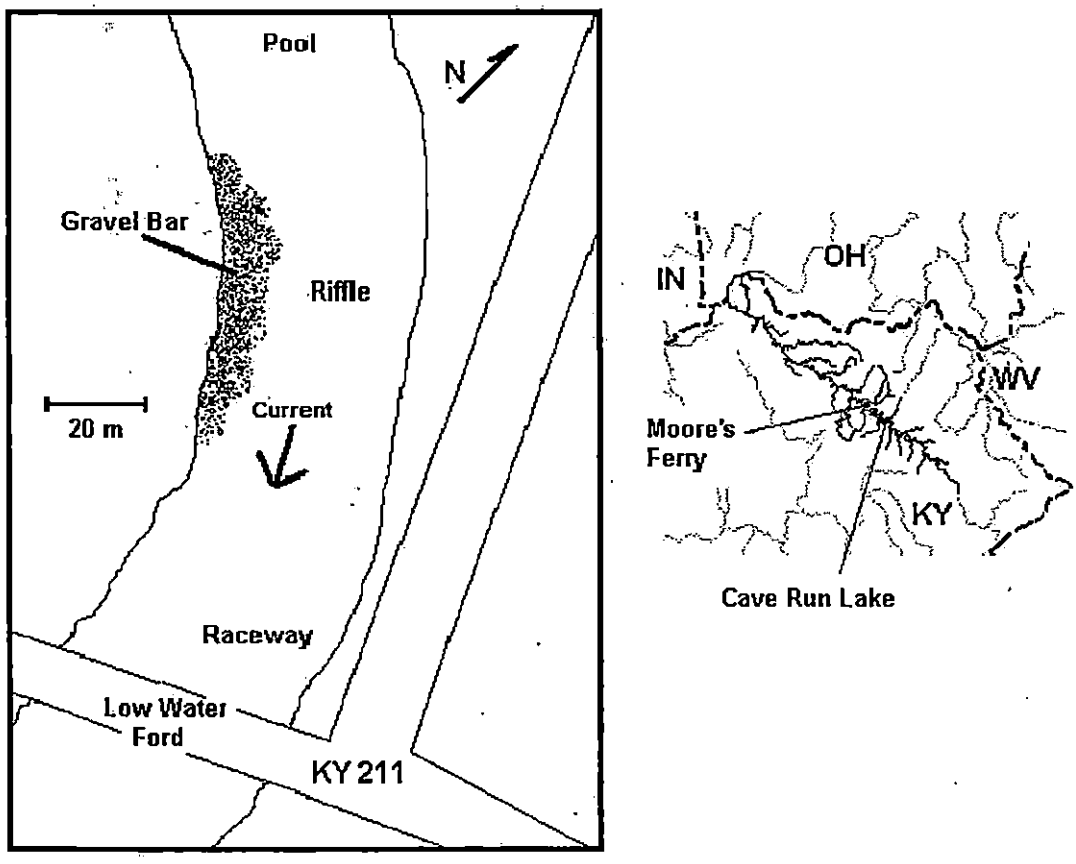


Figure 1. Schematic of the field site at Moore's Ferry, Bath County, Kentucky. Inset: Licking River Basin in Kentucky, illustrating the location of Moore's Ferry and Cave Run Lake.

in some areas in summer. A deep pool is located at the head of the riffle, with a long raceway immediately below the riffle (Figure 1).

General Collections

Madtom collection techniques follow Mayden and Burr (1981) and Burr and Mayden (1982a). Madtoms were collected in areas of likely habitat in the main shoal at Moore's Ferry with 1.6 m X 4.2 m, 2 mm mesh minnow seine from June 19 to November 16, 2001 and June 22 to October 4, 2002. One nighttime collection was made with the headlight gear on September 22, 2002 to document nocturnal activities of *N. stigmosus*. All individuals were measured to the nearest mm SL, and then either released or preserved. A total of 194 *N. stigmosus* were collected at Moore's Ferry, of which 64 were preserved in 10% formalin, transferred to 45% isopropanol, and vouchered in the Morehead State University ichthyological collection (MOSU). Collections could not be made from mid-July through late August 2001 due to flooding from excessive rainfall during this period.

Specimens Examined

Of the 200 individuals of *N. stigmosus* captured during the 2001 and 2002 field seasons, 71 were preserved and deposited in the Morehead State University collection (MOSU). In addition to these specimens, the following vouchered specimens were used in examining the demography, reproductive traits, and general diet of the Moore's Ferry population: MOSU 865, collected 14 July 1984, $n = 1$;

MOSU 630 (P2 clipped), 27 July 1984, $n = 1$; MOSU 866, 31 July 1984, $n = 3$; MOSU 630, 19 July 1985; No catalogue number, 31 July 1985, $n = 2$; MOSU 867, 11 July 1986, $n = 1$; MOSU 868, 1 July 1987, $n = 2$; MOSU 679, 16 July 1988, $n = 13$; MOSU 1014, 3 October 1997, $n = 7$; MOSU 1256, 15 August 1998, $n = 1$.

Demography

Age classes and population structure of the Moore's Ferry population of *N. stigmosus* were determined from length-frequency distributions of individuals captured in 2002 following the methods of Everhart and Youngs (1981) and Macdonald (1987). Length-frequency analyses have been in use since the late 1800s to examine population structures in fish populations. This method is used when the fish being studied is scaleless or has hard anatomical structures that are difficult to obtain or interpret (Everhart and Youngs, 1981). Madtoms are scaleless, their otoliths and fin rays are notoriously difficult to prepare for examination, and removal of fin rays can be injurious to smaller individuals (Burr and Stoeckel, 1999). This study used length-frequency analysis because it provides a quick and easy procedure to collect data, allows for incorporation of data from vouchered specimens, and data can be collected without sacrificing or injuring fish.

The analysis is based on the observation that the lengths of fish of one age tend to form a normal distribution (Everhart and Youngs, 1981). For a large data set, a multimodal distribution may be found, with each mode corresponding to an age class (Royce, 1996). Ages can then be determined by counting the peaks in the

distribution formed by the modes. This is usually adequate for the first two to four years of life, but tends to fail in older age groups because of overlap in the modes resulting from increased dispersion and less distance between modes (Everhart and Youngs, 1981). Thus, the technique is best applied to short-lived species or early stages of longer lived fishes.

The method was modified as suggested by Macdonald (1987) to increase accuracy and reliability of the analysis. Length-frequency relationships are traditionally interpreted visually, but this can lead to inaccurate conclusions, especially in situations where there is much variation in the data or the sample size is small. For accuracy, additional information must be incorporated into the analysis, either through increased sampling, subsampling, imposing biologically reasonable constraints, or using statistical analyses to test for normality within assigned age groups. Of these analyses, maximum likelihood analysis of the modes within the overall length-frequency distributions is the easiest to compute and interpret.

Growth curves were generated for length and gonad development from time-scaled length-frequency data, *sensu* Burr and Mayden (1984). Curves were fitted to weight and length data using ages estimated to the nearest month, assuming a mid-June to mid-July birthdate. Curve-fitting can be a difficult and resource-intensive process, depending on the growth model used. Depending on the application, a simple fit to existing data may be adequate, as long as the residual sum of squares is minimized and the model makes sense biologically (Moreau, 1987). In this study, curve-fitting was adequate in light of the generality of the analysis. For these

analyses, vouchered specimens from Moore's Ferry were pooled with those collected in 2001 and 2002 where appropriate to increase sample size and to preclude negative impacts on the population from extensive sampling.

Catch per unit effort was calculated by dividing total sampling time by number of individuals captured during a given sampling effort. Population density estimates were made by dividing the number of individuals captured by the known area sampled in the habitat study. Population size was estimated by extrapolating density estimates to the entire riffle area.

Quantitative Habitat Survey

Daytime sampling was conducted during the summer and early fall from 26 June 2001 to 20 September 2002. Habitat requirements and habitat micropartitioning between madtoms were examined with the same sampling protocol. Fifteen parallel transects were established across the riffle at Moore's Ferry, along which habitat sampling was conducted. Transects were placed at 10 m intervals, covering the site from the raceway below the riffle to the pool above its head. Along each transect, madtoms were sampled and habitat parameters were recorded at 2 m intervals. Each interval, called a "kick-set", encompassed an area of 2 m^2, approximately the area covered by the seine used in madtom sampling. Three separate sampling events were conducted between 7 September 2001 and 11 August 2002, in which 134 kick-sets were conducted.

At each kick-set, substrate type, current velocity, stream depth, and percent coverage of submerged vegetation, woody debris, and detritus were recorded. Three current velocities were recorded as a 20-second running average at random locations within a kick-set using a Swoffer 3000-C140 current velocity meter. Velocity readings were taken from as close to the substrate as possible without fouling the prop on the substrate or submerged vegetation. Stream depth was measured at five random locations within a kick-set with a meter stick to the nearest 0.5 cm. Substrate type was recorded where the meter stick touched the substrate, and was classified using the size scale given in Barbour et al. (1999). Percent coverage of submerged vegetation, woody debris, and detritus were visually estimated within each kick-set to the nearest 5%.

When all habitat data were recorded, sampling for madtoms was conducted at each kick-set along a transect using the same seine as the general collections. Species, SL to the nearest millimeter, and sex (if discernable) were recorded for all madtoms collected at a kick-set.

Univariate and multivariate analyses of kick-sets were used to examine madtoms distributions within the range of habitat data recorded at Moore's Ferry. The distribution of an organism within *n*-dimensional resource space is the definition of the "niche" (Pianka, 1999), and a nonrandom distribution of an organism along a resource continuum is often considered evidence of the niche (Johnson, 1980). However, the sampling regime of a given study can influence what appears to be niche space based on where the organism is encountered relative to the gradient of the

given resource in the environment, leading to spurious definitions of absolute niche breadth (Colwell and Futuyma, 1971). Therefore, this study will used the term "habitat preference" in describing the range of habitat parameters occupied by groups of madtoms, rather than "niche."

Nesting Survey

Methods for collecting nesting data follow Eisenhour and Burr (2000). Nest searches were conducted by snorkelers in potential nesting habitat above, below, and within the riffle at Moore's Ferry. Dimensions of nest rocks and nest cavities were recorded, and the Barbour et al. (2000) classification system was used to describe nest substrate. Current velocity was measured within the cavity as well as at the level of the cover rock. Madtom larvae or eggs encountered were counted, captured with a dip net, and transferred to the lab for rearing.

Madtom Rearing

Methods were modified from Rakes et al., (2001), Stoeckel and Burr (1999), and Stoeckel and Neves (1996). Fifteen *N. stigmosus* metalarvae collected from Moore's Ferry were kept from 7 July 2002 to 26 July 2002 to document early development. Larvae were maintained in a five-gallon PVC bucket in their native water with native slab rocks and mussel shells provided for cover. Aeration was provided, activated charcoal was added to the substrate to absorb ammonia, and the

water was maintained at ambient room temperature, 21 - 22°C. Larvae were fed finely ground commercial shrimp pellets, which were replaced with fresh food when necessary. Periodically, the larvae were removed and preserved to document development, along with any dead individuals.

Reproductive Traits

Methods for determining reproductive characteristics of *N. stigmosus* followed Mayden and Burr (1981) and Stoeckel and Burr (1999). Gonadosomatic indices (GSI) were calculated for males and females from early summer through mid-autumn using specimens collected in 2001 and 2002 as well as those vouchered in the MSU collection from Moore's Ferry. GSI ratios were calculated as gonad weight divided by adjusted body weight X 1000. Adjusted body weight is the weight of the specimen after the removal of the liver, gut, kidneys, and gonads. All weights were measured to the nearest 0.001 g using a Denver Instruments analytical balance after madtoms and gonads were blotted dry. Seasonality of reproduction and age at sexual maturity were determined from GSI data, along with gonad development and presence of secondary sexual characteristics.

Dissections and examinations of gonads from all specimens were performed under a Nikon SMZ-1B ESD stereomicroscope; reproductive condition was determined by developmental stages of gonads and secondary sexual characteristics. Microscopic examination of ovaries and counting of oocytes allowed for estimates of

relative fecundities of females. Classification of gonad developmental stages follow Stoeckel and Burr (1999) and Burr and Mayden (1982a).

Diet

Methods for determining diet and diel feeding patterns of *N. stigmosus* followed Gutowski and Stauffer, Jr. (1993), Burr et al, (1989), and Hansen et al., (1986). Madtoms were collected and preserved during the daytime on September 20, 2002, and at night on September 22, 2002 to compare diel and nocturnal feeding, gut fullness, and diet of adults and young-of-the-year (YOY) *N. stigmosus*. Individuals of *N. flavus* and *N. miurus* also were collected on these dates to compare diets between syntopic species. Nocturnal collections began approximately 2 hours after sunset to ensure some feeding had taken place, and lasted 2 - 3 hours, until 5 to 10 individuals of each species were collected. The 188 *N. stigmosus* collected during 2001 and 2002 were pooled with specimens vouchered in the MOSU collection from Moore's Ferry for diet description of *N. stigmosus* to preclude mortality from extensive sampling of the Moore's Ferry population.

Guts were removed, dissected, and contents were examined using a Nikon SMZ-1B ESD stereomicroscope. Gut contents were identified to family using Merrit and Cummins (1984), Pennak (1978), and Wiggins (1977). Material collected from madtom guts was preserved in 70% ethanol and deposited in the MSU invertebrate collection.

Statistical Analyses

All statistical analyses were performed with the SAS v.8 and MINITAB 13 desktop software packages. Maximum likelihood analysis was used to support age classes inferred from length-frequency distributions by examining the normality of age classes using the Anderson-Darling normality test. Polynomial regressions were used to fit curves to growth and gonad development data, progressing through linear, quadratic, and cubic functions until the residual sum of squares was minimized.

One-way ANOVA were used to compare habitat variables between YOY and adult *N. stigmosus*, as well as among madtom species. The data set generated during the habitat survey was used, consisting of depth, current velocity, mean substrate size class, substrate variability, and percent coverage of *Potamogeton* recorded at each of 134 kick-sets during the habitat survey. Comparisons of juvenile and adult *N. stigmosus* habitat were made using a partial data set of parameters recorded where the species was present. Comparisons between available habitat and habitat utilization by madtom species were made by comparing habitat variables recorded at each kick-set to the range recorded for each species. Pearson's correlation coefficient (r) was used to further explore the relationship between madtom age classes and habitat utilization with the partial data set.

Principal components analysis was used examine madtom habitat utilization between madtom species and between age classes of *N. stigmosus* in a two-step procedure following Matthews (1985). The original data set of five variables recorded at each of the 134 kick-sets was reduced into principal components extracted

from a correlation matrix of the original data. This allowed the classification of kick-sets based on the habitat parameters recorded for each. Madtom presence and absence then was plotted along the PC axes so presence of absence of each species could be correlated with the combination of original habitat variables underlying the multivariate axes (Matthews, 1998). The same procedure was followed in examining habitat use between *N. stigmosus* age classes with a partial data set containing only sites where the species was present. In both multivariate analyses, overlap in multivariate space was interpreted as an indication of potential competition, while segregation was interpreted as evidence for resource partitioning (Matthews, 1985).

RESULTS

Associated Species

While snorkeling, species observed in association with *N. stigmosus* were: *N. miurus, N. flavus, Etheostoma blenneoides, E. camurum, E. flabellare, E. variatum, E. zonale, Percina caprodes, P. evides,* and *P. copelandi.* Additional species were commonly captured with *N. stigmosus* while seining, including *Notropis rubellus, Notropis volucellus, Hypentelium nigricans, Cyprinella whipplei, Pimephales notatus,* and *Nocomis micropogon* (Table 1).

Table 1. Frequency of fish species observed while snorkeling or collected during seining in the Licking River at Moore's Ferry, 2001-2002.

Species	Snorkel	Seine
Clupeidae		
Dorosoma cepedianum	0	1
Cyprinidae		
Campostoma anomalum	0	5
Cyprinella spiloptera	0	1
C. whipplei	0	9
Macrhybopsis hyostoma	0	1
Nocomis micropogon	0	7
Notropis atherinoides	0	1
N. buccatus	0	1
N. ludibundus	0	3
N. rubellus	0	7
N. volucellus	1	5
Pimephales notatus	0	6
Hypentelium nigricans	1	6
Ictaluridae		
Ictalurus punctatus	0	4
Noturus flavus	2	12
N. miurus	1	10
N. stigmosus	3	11
Atherinidae		
Labidesthes sicculus	0	1
Centrarchidae		
Ambloplites rupestris	0	2
Lepomis macrochirus	0	2
L. megalotis	0	1
Micropterus punctulatus	0	1

Table 1. (Cont.)

Species	Snorkel	Seine
Percidae		
Ammocrypta pellucida	0	1
Etheostoma blennioides	3	11
E. caeruleum	0	2
E. camurum	4	10
E. flabellare	4	10
E. nigrum	0	4
E. spectabile	0	1
E. variatum	3	11
E. zonale	4	11
Percina caprodes	1	3
P. copelandi	4	11
P. evides	2	9
P. oxyrhyncus	0	2
Total sampling events	**7**	**13**

Demography

Estimates of population density and population size fluctuated widely from 2001 to 2002. Based on 309 individual seine kicks, population density at Moore's Ferry was estimated at one individual per 47.7 m^2 for 2001, and one individual per 3.4 m^2 for 2002. Extrapolating to the approximately 4500 m^2 of available habitat at the site, the population of *N. stigmosus* was estimated at 95 individuals for 2001 and 1325 individuals for 2002.

Examination of the length-frequency histogram of all specimens captured in 2002 (Figure 2) and comparison to published accounts for other closely related species (Burr, et al., 1989; Starnes and Starnes, 1985; and others) indicates at least three age classes are present; age class 0 (0-12 months; $\leq$ 50 mm SL), age class 1 (12-24 months; 53 - 61 mm SL), and age class 2+ (> 24 months; $\geq$64 mm SL). The designation age class 2+ is used to indicate likely presence of fish older than two years of age in the group. Variation in the YOY data and overlap between the modes of older fish make interpretation more difficult, so maximum likelihood analysis and monthly subsampling of the length-frequency distribution were used to validate the age class assignments.

Maximum likelihood analysis supports the age class 1 and age class 2+ groups, as both are normally distributed ($A^2 = 0.592$, $p = 0.110$ and $A^2 = 0.571$, $p = 0.119$, respectively) (Figure 3). However, the age class 0 group is not normally distributed ($A^2 = 7.069$, $p = 0.000$), but this is most likely because the group is made

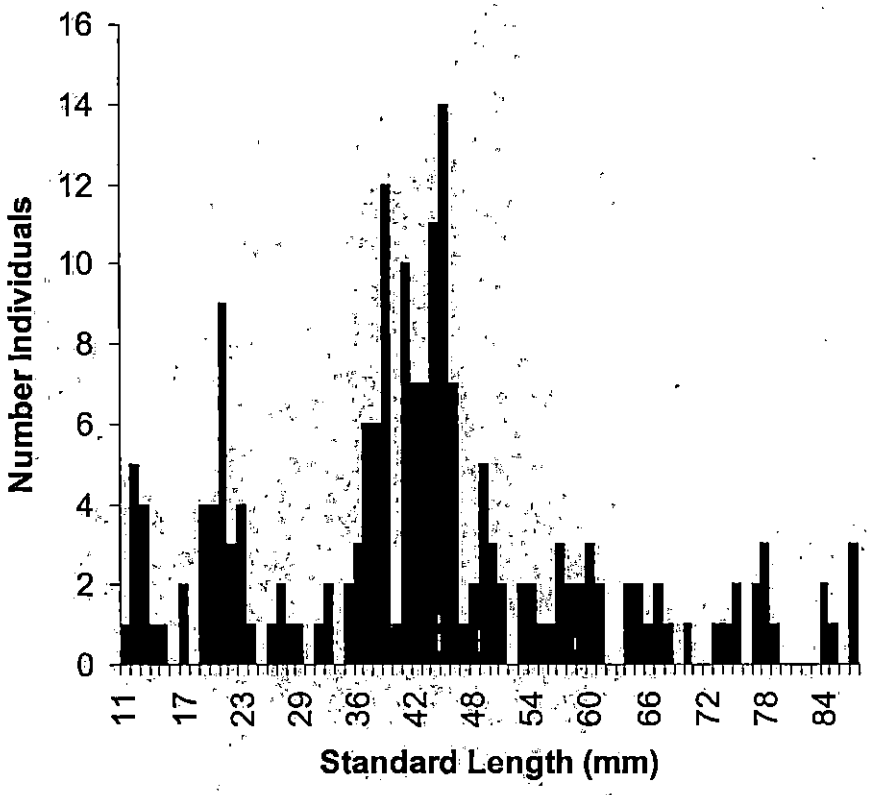


Figure 2. Length-frequency histogram generated from 188 *N. stigmosus* collected from Moore's Ferry, 26 June to 4 October 2002.

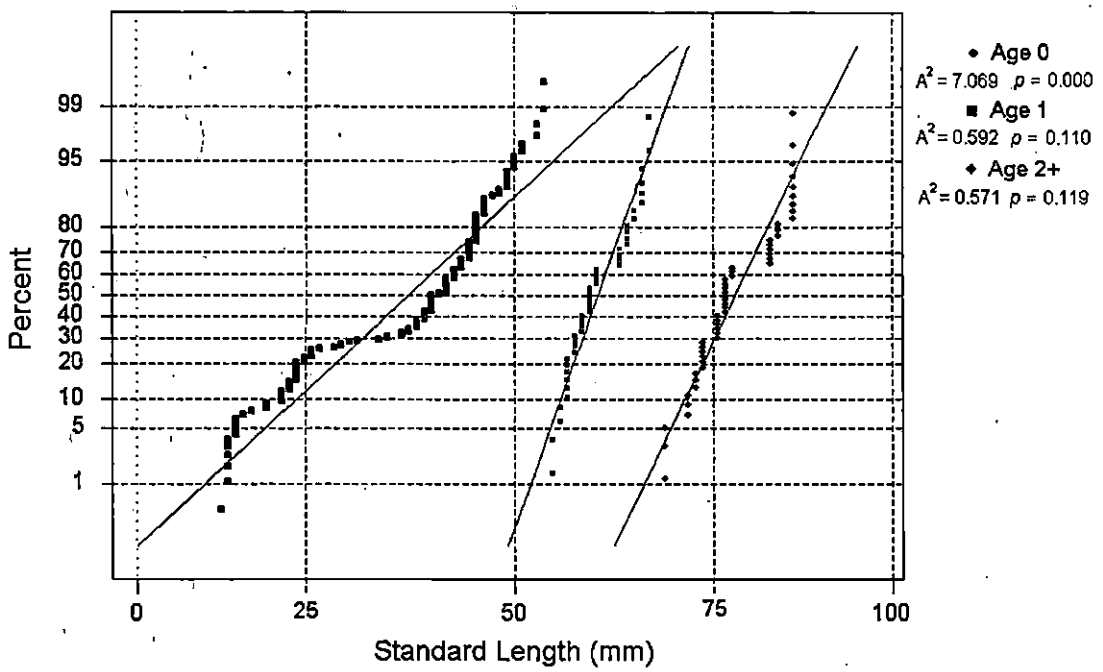


Figure 3. Maximum likelihood analysis of age classes of *N. stigmosus* inferred from length-frequency distributions of 188 individuals collected from 26 June to 4 October 2002.

up of several separate clutches at various stages of development, when growth is especially rapid.

A histogram of individuals captured in July 2002 provides some clarification to the age class assignments (Figure 4). A distinct mode is seen corresponding to age class 1 fishes, with another less distinct mode of age class 2+ fish. YOY still appear to be bimodal, likely reflecting different dates of capture of rapidly growing juveniles. The first group, 11 – 14 mm SL, were captured in early July when a nest was discovered. The second group, 18 – 25 mm SL, were collected later in the month after the cohort had grown larger.

A histogram of the lengths of fish captured in September 2002 indicates that YOY remain below 50 mm SL (Figure 5). A clearly defined mode from approximately 35 mm SL to 48 mm SL shows the fish spawned in July nearing the end of their summer growing season; madtom growth is negligible over the winter (Burr and Stoeckel, 1999). Despite the lack of continuity in the mode for YOY in the overall 2002 length-frequency distribution, it is clear that they remain under 50 mm SL until the end of the first year of life.

Assuming that each age class was caught in proportion to its abundance at Moore's Ferry, in 2002 the population was made up of 80.3% age 0, 11.2% age 1, 8.5% age 2+ fish (Table 2). Relative survival values were calculated for each age class of *N. stigmosus*, with 13.9% of young-of-the-year (YOY) surviving to their second summer, and 10.6% of the original cohort surviving to age 2+. While YOY survival is low, 76.2% of age 1 fish survive to age 2+. This also assumes no

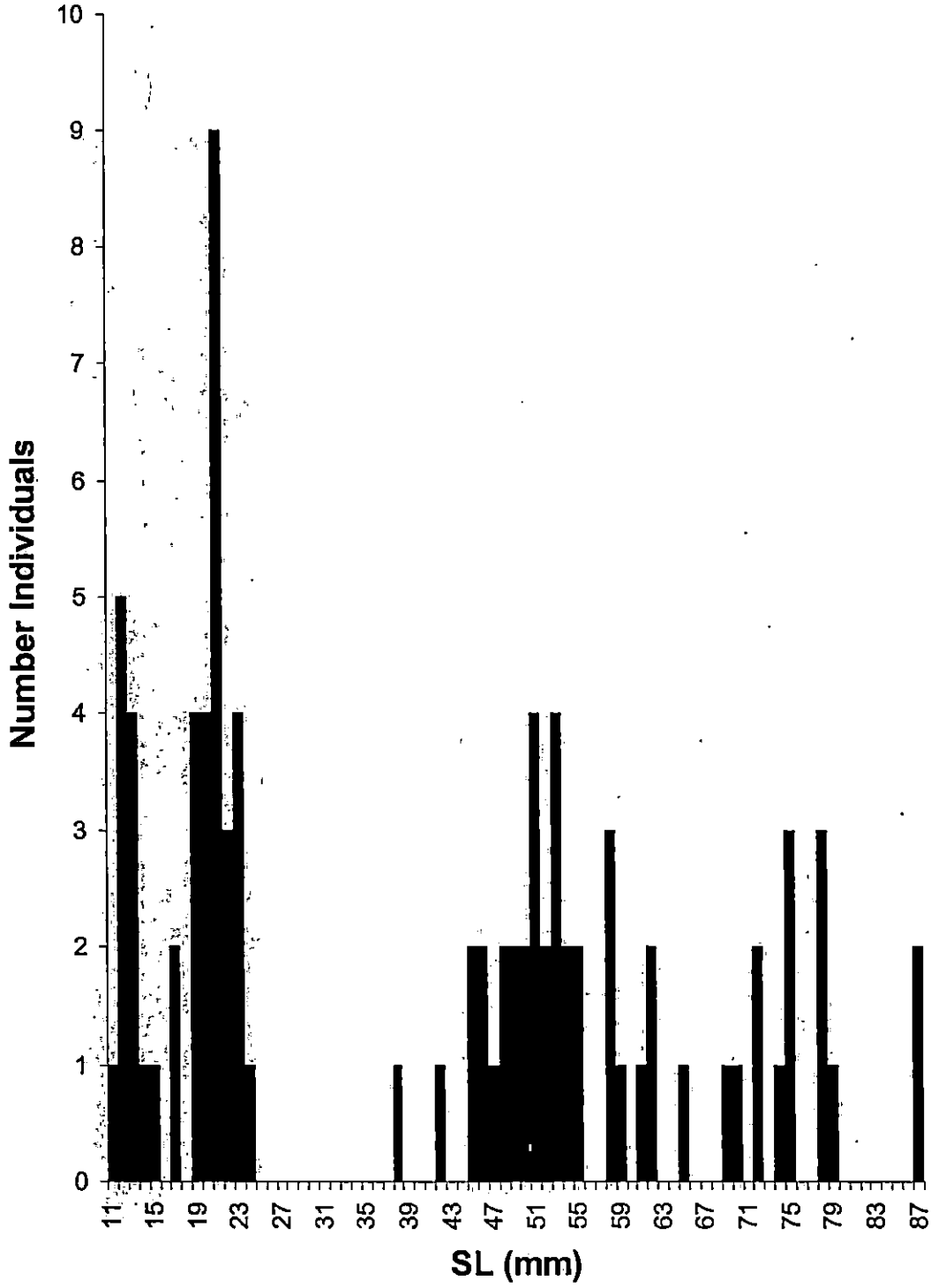


Figure 4. Length-frequency histogram of 88 *N. stigmosus* collected in July 2002.

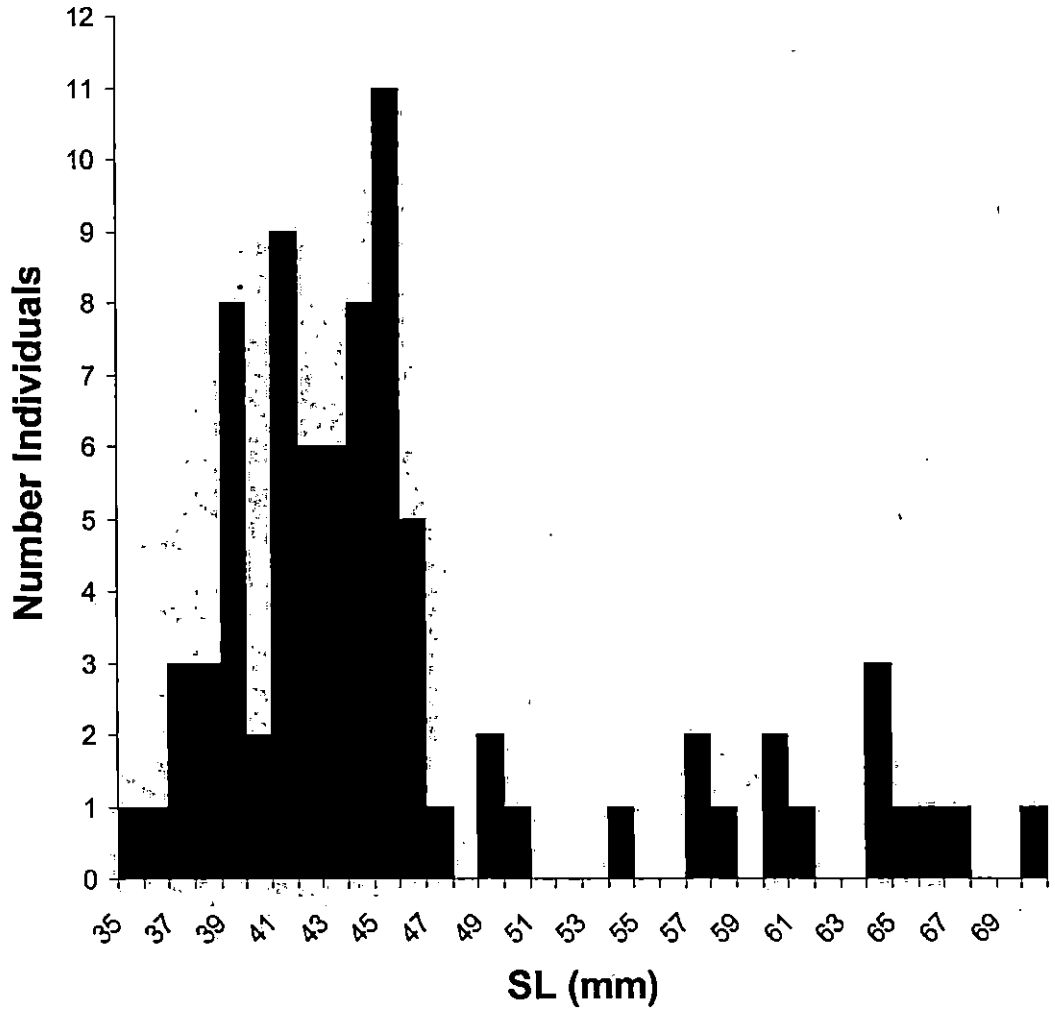


Figure 5. Length-frequency histogram of 81 *N. stigmosus* collected in September 2002.

Table 2. Distribution and survival of age classes of *N. stigmosus* in 2002 at Moore's Ferry, expressed as proportions of age class 0 ($1X^1$) and age class 1 ($1X^2$).

Age Class	Number of Individuals	Survival	
		$1X^1$	$1X^2$
0	151	1.000	---
1	21	0.139	1.000
2+	16	0.106	0.762
Total	**188**		

migration of adult fish into the Moore's Ferry population, which is reasonable in light of the unsuccessful attempts to collect *N. stigmosus* from several other localities.

Growth

Growth rate for *N. stigmosus* was determined from curves fitted to weight and length measurements at ages estimated from the length-frequency analysis. There was no significant difference between male and female growth rates for length, so data were pooled. The relationship was best described by the regression equation $L = 23.26 + 3.28A - 0.05A^2$, $r^2 = 0.797$ (Figure 6). Three individuals attained maximum length recorded at Moore's Ferry, 87 mm SL. The monthly length-frequency relationship for *N. stigmosus* captured during 2001-2002 (Figures 4 and 5) illustrates the rapid growth of YOY. Young-of-the-year madtoms collected in July ranged from 11 to 38 mm SL, and had attained a size of 35 to 47 mm SL by September. Individuals attain 55 - 60 mm SL by one year and 65 - 70 mm SL by two (Figure 2).

The relationship between adjusted body weight and length for *Noturus stigmosus* is shown in Figure 7. Both males and females showed a linear increase in weight with length, best described by the equation $W = -6.93 + 1.88L$, $r^2 = 0.934$ in males and $W = -4.35 + 0.140L$, $r^2 = 0.967$ in females. Females were slightly heavier than males until they reached approximately 50 mm SL, when the male growth rate overtook the female growth rate. Maximum adjusted weights for females and males, respectively, were 6.61 g and 9.97 g.

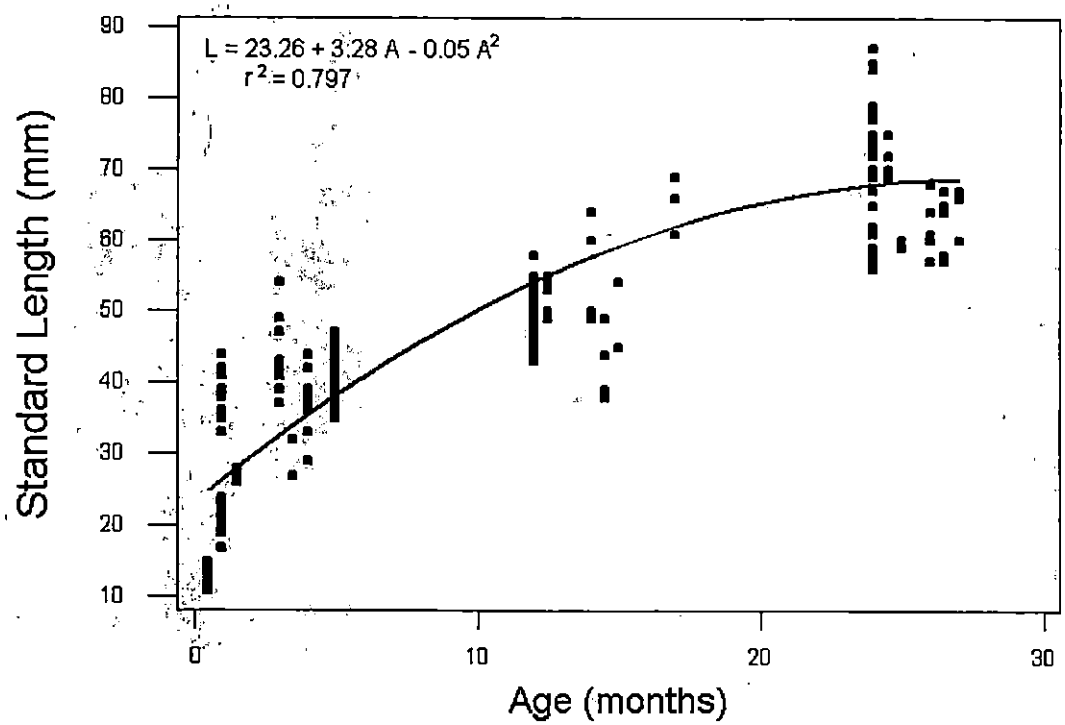


Figure 6. Relationship between length and age for 239 *N. stigmosus* collected from Moore's Ferry.

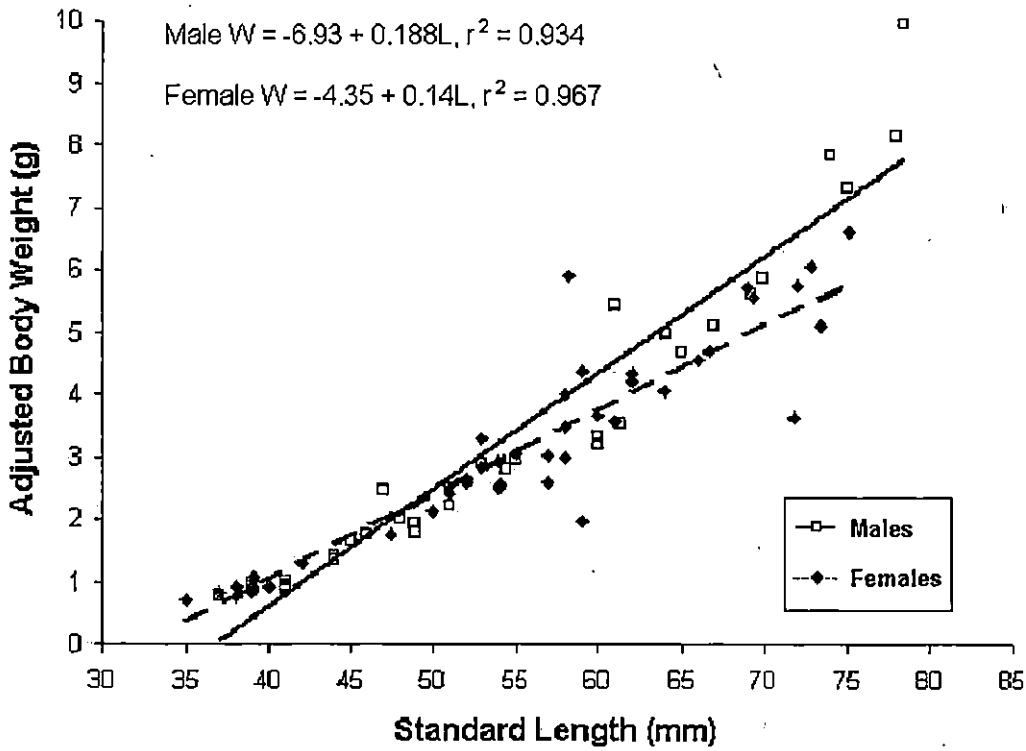


Figure 7. Relationship between adjusted body weight and standard length for 35 male and 47 female *N. stigmosus* at Moore's Ferry.

Male Reproductive Cycle

Testes of male *Noturus stigmosus* were similar in structure and appearance to published descriptions of other ictalurids and madtom species (Stoeckel and Burr, 1999; Burr et al., 1989; Burr and Mayden 1982a; and others). Mature testes were elongate structures, opaque white to creamy yellow in color, with numerous fingerlike projections of various sizes. Immature testes dissected from age class 0 fishes were smaller, clear, and had fewer, smaller projections.

Gross examination of males collected from 1984 to 2002 showed that testis development gradually increased as fish grew larger. Gonadosomatic indices (GSI) for these individuals are low in age class 0 individuals (under 60 mm SL), but reach an inflection point and begin to rise quickly at 60 mm SL, at about twelve months of age (Figure 8). Gonadosomatic indices for adult males are high in late June, peak in mid-July, and then drop in mid-August, remaining low through mid-autumn (Figure 9).

Reproductive males captured from late June through July showed well-developed secondary sexual characteristics. Specimens had swollen lips, cephalic epaxial muscles, and genital papillae typical of breeding male madtoms. Genital papillae of reproductive males were larger and more elongate than those of nonbreeding males. In addition to these characters, reproductive males showed enhanced coloration. The overall cast of the body was orange-yellow, with marked areas of pink on the venter and underside of the head, especially the lips. The fins had an overall yellow cast with orange tips. In particular, the pectoral fins bore a

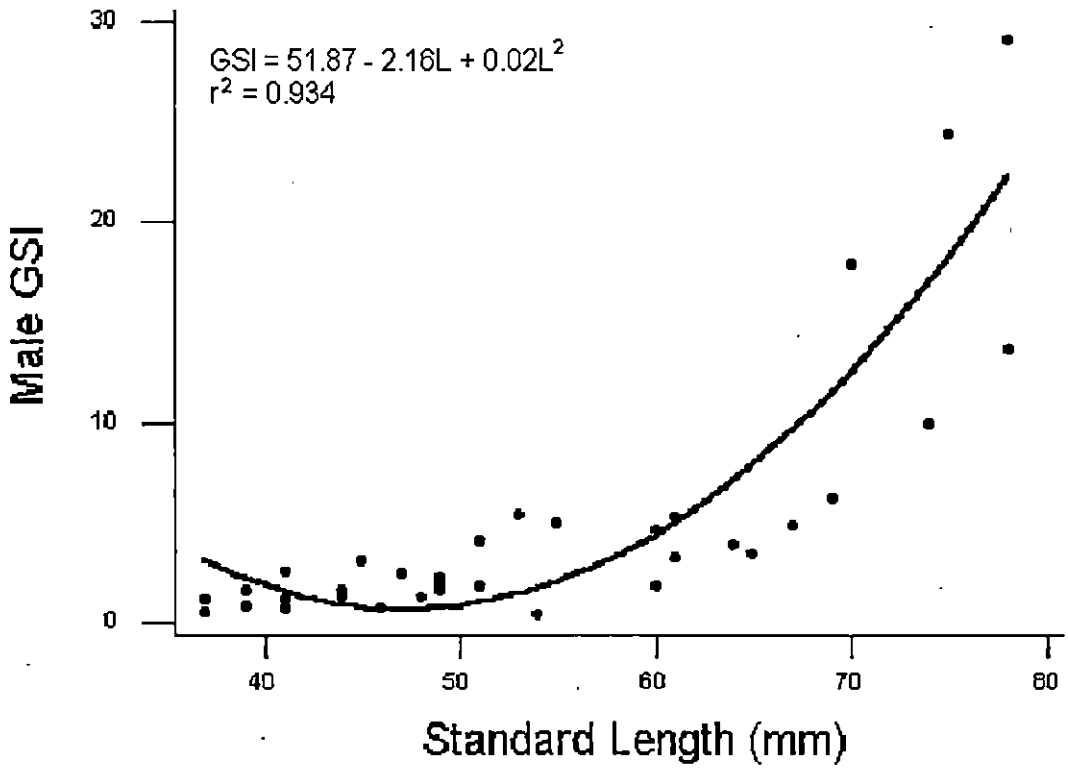


Figure 8. Relationship between gonadosomatic index and standard length for 35 male *N. stigmosus* collected from Moore's Ferry.

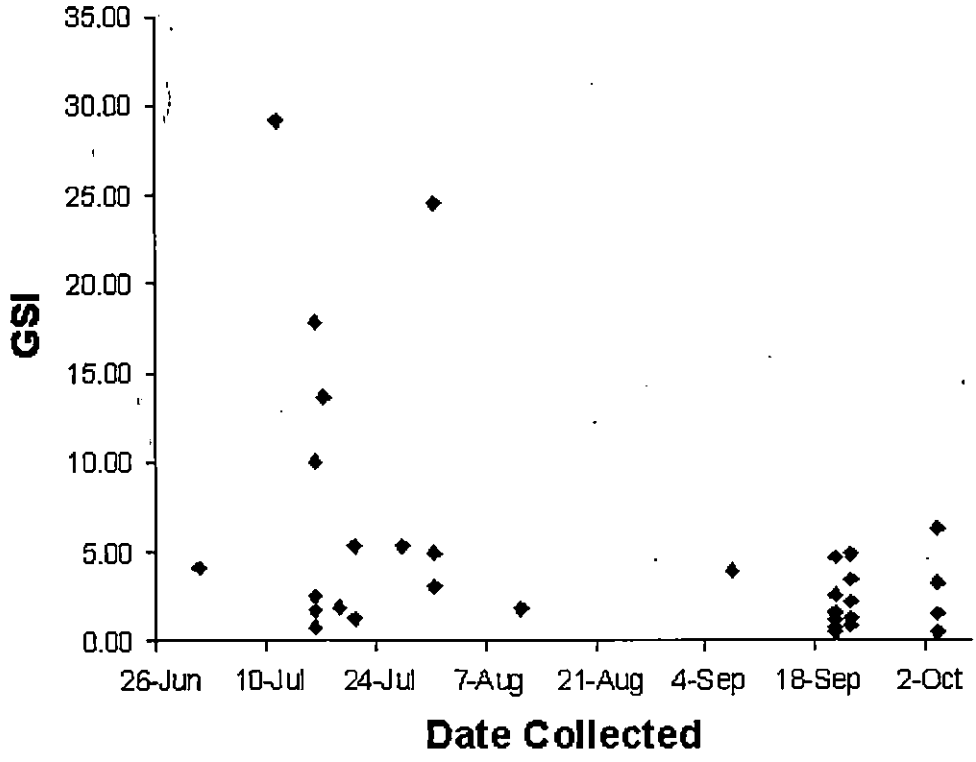


Figure 9. Seasonal variation in gonadosomatic index for 35 male *N. stigmosus* collected from Moore's Ferry.

yellow cast fading to pink in the distal third of the leading edge. No males under 60 mm SL exhibited well-developed secondary sexual characteristics during the breeding season.

Female Reproductive Cycle

Ovaries of female *N. stigmosus* were oblong to kidney shaped, similar in structure and appearance to descriptions published for other madtom species (Stoeckel and Burr, 1999; Bur et al., 1989; Burr and Mayden, 1982a; and others). Sexually mature individuals could be distinguished readily from immatures by the relative size and appearance of the ovaries. Immature ovaries contained one size class of oocytes, which were small in diameter (0.1 – 0.5 mm), spherical, and opaque white to creamy yellow in color. Mature ovaries were larger than those from immature specimens, with two distinct size classes of oocytes. Resting oocytes were smaller in diameter (1.2 – 2.3 mm, mean = 1.2 mm), and similar in color to immature oocytes, while mature oocytes were larger (1.0 – 3.1 mm, mean = 2.2 mm), spherical to polygonal in shape, and ranged from yellow-orange to amber in color.

Females captured during the spawning season from 1984 to 2002 showed that gonad development was insignificant until individuals reached approximately 60 mm SL. Gonadosomatic indices were low for the fish in age class 0 (< 50 mm SL), but rose as females reached approximately one year of age (55 – 58 mm SL) (Figure 10). This relationship is similar to what was seen in males, but the relationship was not as well-defined for females ($r^2 = 0.934$ and $r^2 = 0.452$, respectively). Outliers from the

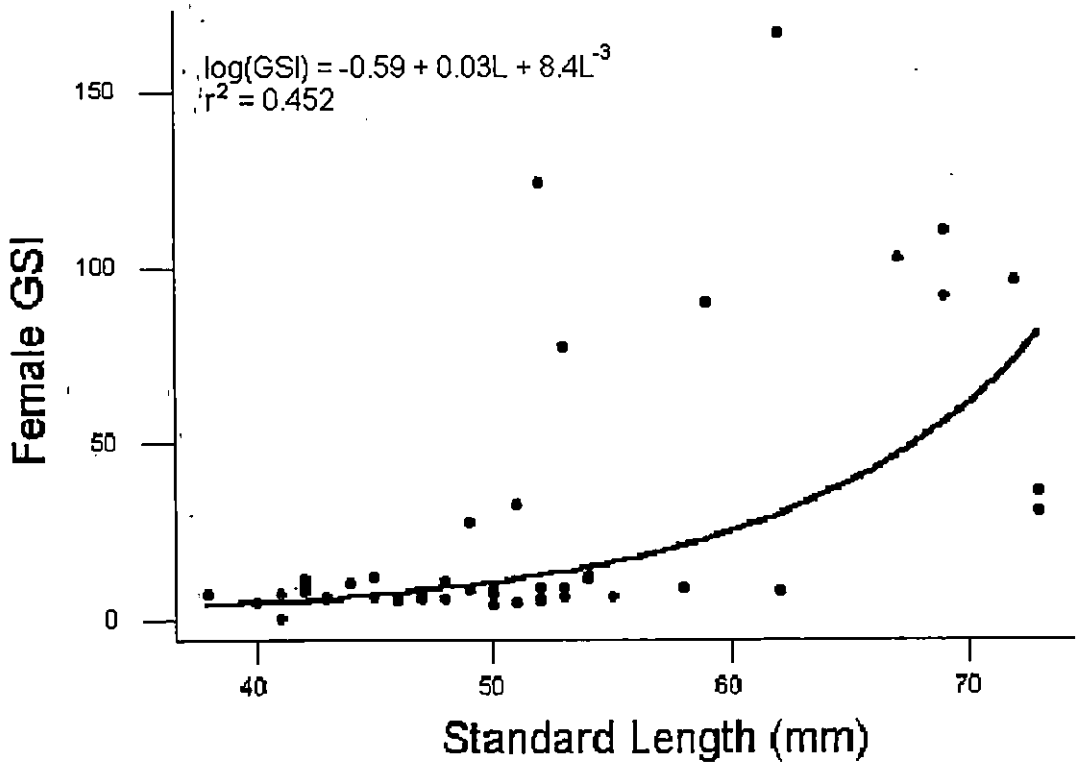


Figure 10. Relationship between gonadosomatic index and standard length for 47 female *N. stigmosus* collected from Moore's Ferry.

fitted curve are likely responsible for the error not accounted for by the regression equation. Three females greater than 60 mm SL have low GSI values relative to other females of similar lengths, and probably represent fish captured after spawning has occurred. There were also two smaller individuals (~55 mm SL) that had high GSI values, but had no distinct mature oocytes in their ovaries. However, these individuals did have oocytes larger than those typical of YOY, and may represent females that are reaching reproductive potential early in life. The seasonal variation of GSI values reflects the pattern seen in males; gonadosomatic index values in females rise in mid-June, peak in mid-July, and fall rapidly in late July (Figure 11).

Females collected during the spawning season exhibited secondary sexual characteristics typical of other species in genus *Noturus*. Females had swollen genital papillae and distended abdomens, as well as enhanced coloration that was similar to reproductive males; however, the papillae of the females were wider and blunter than those of males, and the coloration was less intense. Females less than 58 mm SL did not exhibit secondary sexual characteristics during the spawning season.

In the ten reproductively mature females examined, the number of mature oocytes present per female ranged between 34 and 98 (mean = 76.9) (Table 3). The largest female, in both length and weight produced the most oocytes. However, the number of oocytes was only correlated with adjusted weight ($r = 0.730$; $p = 0.017$), not with age ($r = 0.492$; $p = 0.149$) or length ($r = 0.517$; $p = 0.126$). Ages were estimated at 2 and 3 years based on the large range in size of age class 2+ fish determined from the length-frequency analysis and from published accounts of

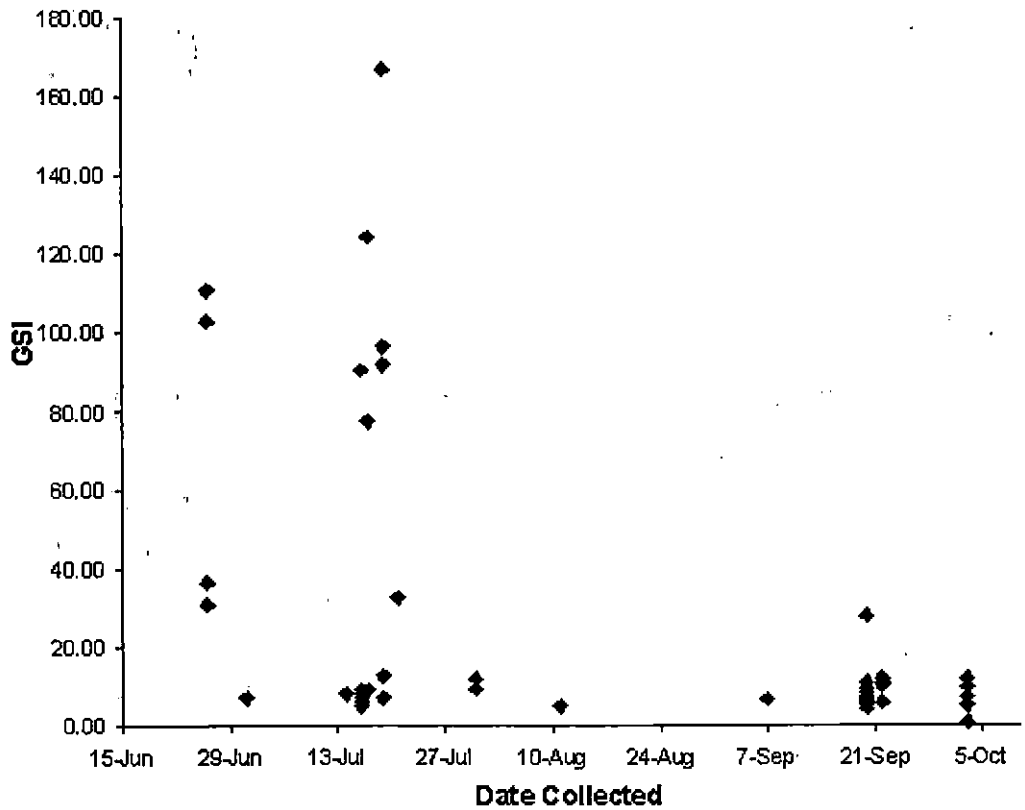


Figure 11. Seasonal variation in gonadosomatic index for 47 female *N. stigmosus* collected from Moore's Ferry.

Table 3. Relationship between size, age, ovary weight, and the number of mature oocytes in 10 reproductively mature *Noturus stigmosus* females at Moore's Ferry.

SL (mm)	Adjusted Body Weight (g)[a]	Month of Collection	Age in Years	Weight of Ovaries (g)	Number of Mature Oocytes	GSI[b]
58	3.49	July	2	0.11	34	33
58	5.90	July	2	0.73	76	124
59	4.37	July	2	0.39	81	90
62	4.23	July	2	0.71	82	167
69	5.55	June	3	0.62	86	111
69	5.74	July	3	0.52	83	92
72	5.75	July	3	0.55	66	96
73	5.10	June	3	0.15	71	31
73	6.05	June	3	0.23	92	37
75	6.61	July	3	0.51	98	77

[a]Adjusted body weight is the specimen's weight after removal of the gonads, stomach, intestine, and liver

[b]Equals weight of ovaries x 1000/adjusted body weight

N. stigmosus and other species. It appears that weight is a better predictor of individual female fitness than either length or age, a pattern reflected in the pair of females at 58 and 73 mm SL in Table 3. In both cases, the heavier female for the same length produced more oocytes.

Nesting Survey

Snorkeling surveys accounting for 16.5 person-hours of effort were conducted at Moore's Ferry from June 9 to July 17, 2001, and 8 person-hours were spent between June 22 and July 7, 2002. One nest and two potential nesting sites were discovered for *N. stigmosus* during this time, as well as one *N. flavus* nest.

On July 7, 2002, a nest of approximately 40 *N. stigmosus* larvae was discovered in 40 cm deep water at the head of the riffle at Moore's Ferry. The nest was located in a cavity 25 cm x 27 cm x 7.5 cm deep below a 46 cm x 2.5 cm x 5 cm slab rock in an area of mixed gravel and cobble. Current velocity within the cavity was 0.28 m/sec with the nest rock removed, and 0.49 m/sec in the water column above the rock. Substrate consisted of sand and some fine gravel within the cavity, and ambient water temperature was 25°C. No guardian adult was present at the nest. Nineteen of the 40 observed larvae were captured; four of these were immediately preserved with the rest transported to the lab for examination and rearing. Several

other larvae fled from the nest when it was disturbed; many of these individuals were eaten by *Notropis volucellus* and *Etheostoma blennioides.*

Based on Burr and Mayden's description of juvenile *N. nocturnus* (1982b), the *N. stigmosus* captured on 7 July were metalarvae, at an estimated age of two weeks. Madtoms at this stage have just absorbed the yolk sac, which was apparent as a small yellow area on the posterior of the venter of *N. stigmosus* larvae. Metalarvae all possessed well-developed barbels, cephalic pore systems, lateral lines, and fin rays, and showed development of adult coloration.

An attempt to rear the remaining 15 larvae was made for two weeks. Survival was comparable to accounts from captive propagation of other species (Rakes et al., 2001; Stoeckel and Neves, 1996), however, the larvae did not readily feed and became emaciated. After losing significant amounts of weight, the survivors at two weeks were preserved.

Two potential nest sites were discovered in the summer of 2001. On 19 June, a 77 mm SL male *N. stigmosus* with developing secondary sexual characteristics was discovered in a depression underneath a 20 cm x 30 cm slab rock in 40 cm deep water in the main body of the riffle at Moore's Ferry. Water temperature was 21°C, and the current velocity was 0.33 m/sec. A male *N. stigmosus* approximately 50 mm SL was found on 26 June below a 34 cm x 29 cm slab rock in 28 cm deep water at 22°C with a current velocity of 0.16 m/sec.

On 26 June 2001, a 129 mm SL male *N. flavus* was found guarding a clutch of 95 eggs in 30 cm deep water with a current velocity of 0.65 m/sec. The nest was

located beneath a 56 cm x 42 cm x 6 cm slab rock; current velocity in the nest cavity was 0.072 m/sec. This egg mass was brought back to the lab and maintained in an attempt to rear the embryos. After a few days, fungal growth had covered much of the egg mass, which was then preserved and deposited in the Morehead State University collection.

Habitat Utilization

Available habitat was considered to be the area sampled that fell within recorded habitat preferences for a given madtom species; utilized habitat was the area actually occupied by madtoms. Areas for each species do not sum to the total area surveyed due to overlap.

Of 282 m^2 of potential habitat surveyed, over half (148 m^2) was available for madtoms. The majority of available habitat fell within *N. stigmosus* preferences (116 m^2), followed by *N. miurus* (80 m^2), and *N. flavus* (52 m^2) (Table 4). Over 50% of available habitat was suitable for multiple madtom species. Madtoms occupied less than 30% of the available habitat for each species, and only 5% of habitat suitable for multiple species was utilized by more than one madtom.

Intraspecific Habitat Preferences of Noturus stigmosus

Noturus stigmosus was most common in areas with current velocities of approximately 0.5 m/sec, 20 - 23 cm depth, moderate substrate sizes, and less than 40% coverage of *Potamogeton*.

Table 4. Available habitat and utilization by madtom species at Moore's Ferry, based on ranges of habitat parameters recorded for each species at 134 kick-sets during quantitative habitat survey.

Species	Potential Habitat	Habitat Occupied	Percentage Occupied
N. stigmosus	116 m^2	24 m^2	20.6
N. miurus	80 m^2	12 m^2	15.0
N. flavus	52 m^2	14 m^2	26.9
Multiple Species	80 m^2	4 m^2	5.0

Individuals of *N. stigmosus* showed few differences in habitat utilization between age classes. Based on one-way ANOVAs, there were no significant differences in habitat variables between juvenile ($n = 9$) and adult ($n = 8$) fish (current velocity, $p = 0.165$; mean substrate size class, $p = 1.000$; depth, $p = 0.331$; vegetative coverage, $p = 0.228$) (Figure 12). However, a Pearson correlation did show a significant positive correlation between depth and increasing age ($r = 0.675$, $p = 0.003$) and a significant negative correlation between vegetative coverage and increasing age ($r = 0.584$, $p = 0.014$). Young-of-the-year also were frequently observed in areas of slower current at the head of the riffle at Moore's Ferry.

Principal components analysis extracted five axes from the original habitat variables, the first three of which accounted for 91.1% of the variance (Table 5). The first axis, principal component 1 (PC 1) accounted for 47.6% of the total variance.

Four variables on PC 1 had absolute loadings of > 0.40, corresponding to current velocity (0.569), depth (0.584), and percent coverage of *Potamogeton* (0.559). Principal component 2 accounted for another 22.7% of the total variance, while PC 3 accounted for another 20.9%. However, these axes are somewhat less informative than PC 1 because they only have one variable apiece with an absolute loading > 0.40, corresponding to substrate variability (0.882) and mean substrate size class (0.945), respectively.

A scatterplot of *N. stigmosus* presence overlaid on the principal component axes of habitat preferences is given in Figure 13. There was great overlap of clusters for YOY and adult fish, with a tight cluster for YOY within adult habitat preferences.

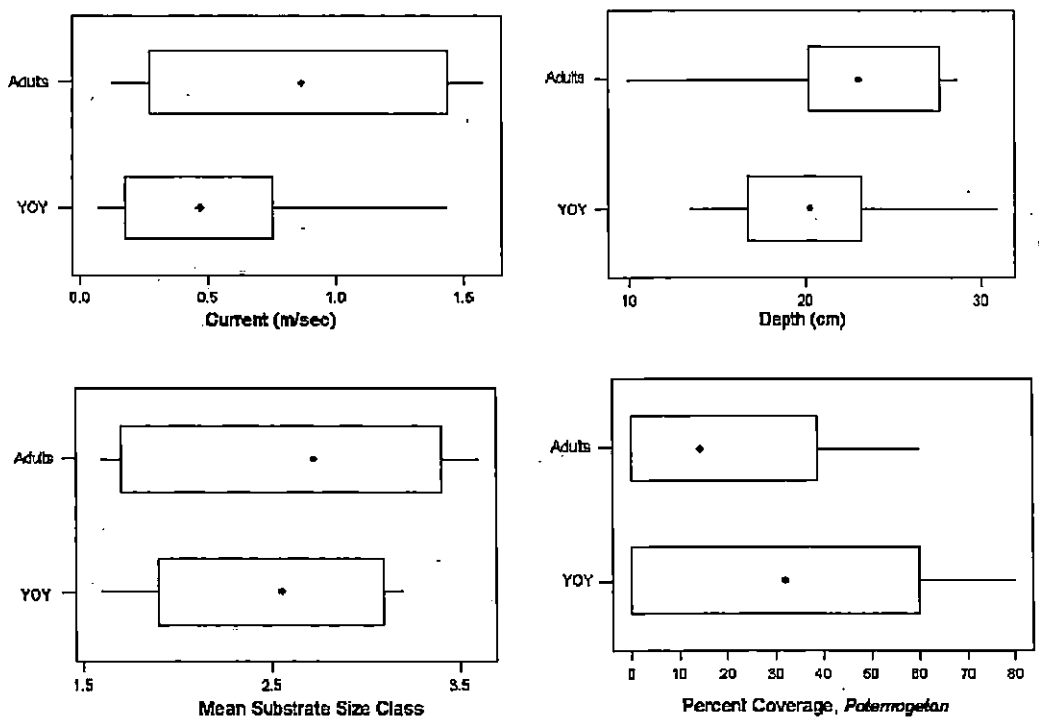


Figure 12. Comparison of habitat utilization between YOY (n = 9) and adult (n = 8) *N. stigmosus* in the Licking River at Moore's Ferry based on one-way ANOVA of madtom age class versus habitat variables. Bars represent 95% confidence intervals, horizontal lines the range of values recorded for each age class, and dots the means.

Table 5. Eigenvalues, proportion of variance, and loadings for principal components analysis of 5 habitat variables from 17 kick-sets containing YOY and adult *N. stigmosus* at Moore's Ferry, Licking River, Kentucky.

	PC1	PC2	PC3
Eigenvalue	2.379	1.331	1.043
Proportion of total variance	0.476	0.227	0.209
Eigenvectors:			
Current Velocity	0.5688	0.2929	-0.0952
Substrate Size	-0.1059	-0.1388	0.9455
Substrate Variability	0.1080	0.8819	0.2399
Depth	0.5843	-.22579	0.0324
Percent Coverage, *Potamogeton*	-0.5588	0.2252	-0.1958

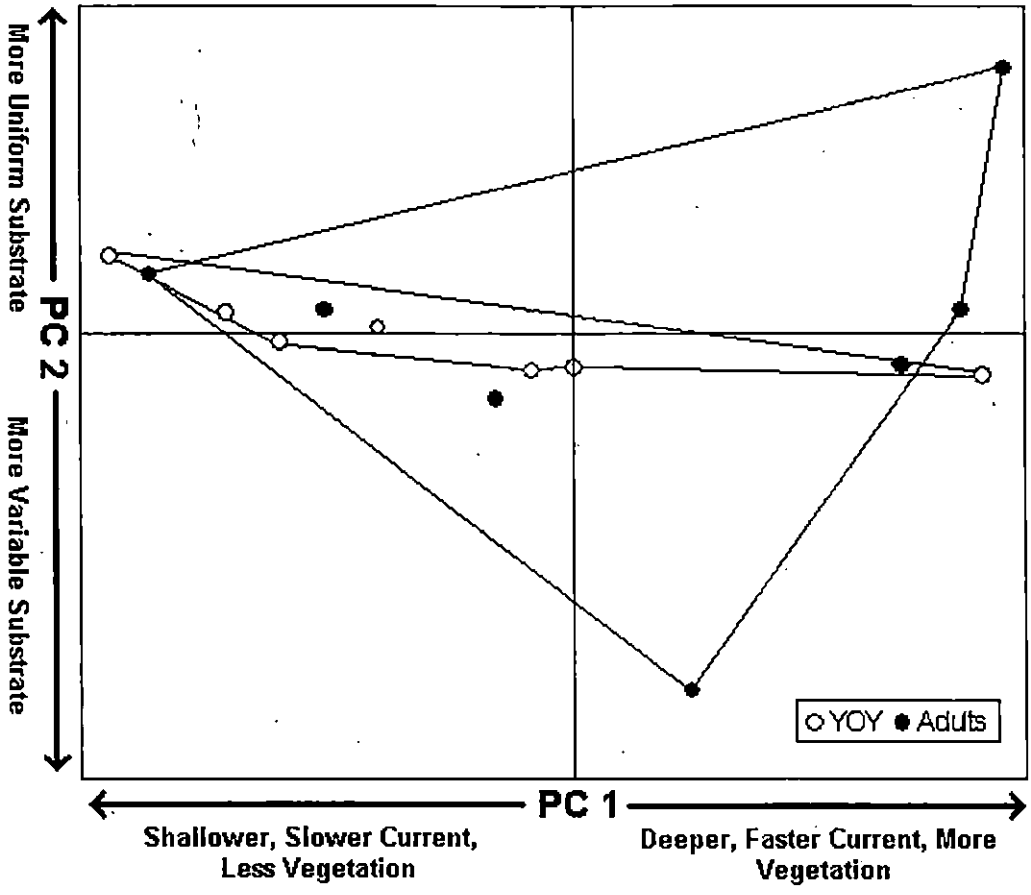


Figure 13. Scatterplot of 17 kick-sets at Moore's Ferry containing YOY and adult *N. stigmosus* on multivariate axes generated by PCA.

Juveniles were grouped tightly on the left axis of PC 2, corresponding to current velocity, depth, and percent coverage of *Potamogeton*. This suggests juvenile *N. stigmosus* may occupy a narrow range within adult habitat preferences, and were most common in areas of shallower water, slower current velocity, and greater vegetation.

Interspecific Madtom Habitat Preferences

There were marked differences in habitat utilization between the three species of madtoms at Moore's Ferry. One-way ANOVA showed *N. stigmosus* ($n = 17$) was found in areas with deeper water ($p = 0.028$), faster current ($p = 0.048$), and less vegetation ($p = 0.046$) than *N. flavus* ($n = 11$). The two species did not differ in mean substrate size or substrate variability. Habitat preferences for *N. miurus* (n = 6) were not significantly different from either *N. flavus* or *N. stigmosus* (Figure 14).

Principal components analysis extracted five axes from the original variables. The first three axes accounted for 83.6% of the variance in the data (Table 6). Principal component 1 had three variables that had absolute loadings >0.40, corresponding to current velocity (0.584), depth (0.590), and vegetative coverage (0.553) (Table 6). Principal component 2 and 3 both had two variables with absolute loadings >0.40, corresponding to mean substrate size and substrate variability.

A scatterplot of all 134 kick-sets overlaid on multivariate axes generated from PCA shows that madtoms as a whole occupied areas with greater current velocities and larger substrates relative to what was present at Moore's Ferry (Figure 15).

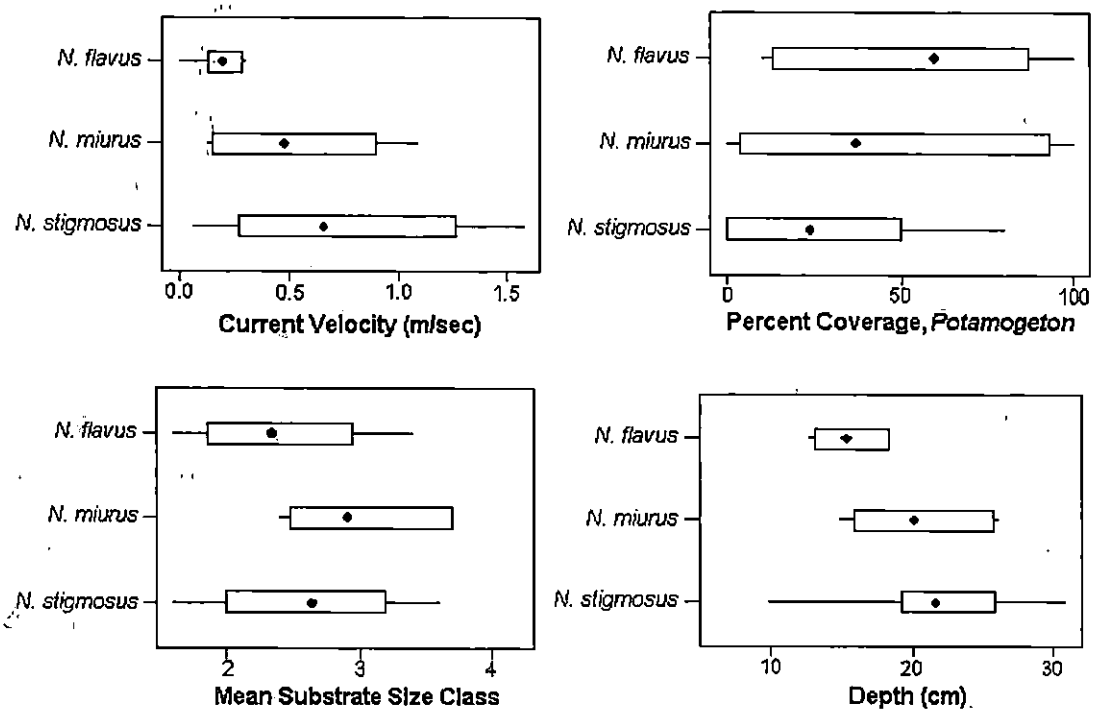


Figure 14. Comparison of habitat utilization between *N. stigmosus* (n = 17), *N. flavus* (n = 11), and *N. miurus* (n = 6) in the Licking River at Moore's Ferry based on one-way ANOVA of madtom species versus habitat variables. Boxes represent 95% confidence intervals, horizontal lines the range of values recorded, and dots the means for each species.

Table 6. Eigenvalues, proportion of variance, and loadings for principal components analysis of 5 habitat variables from 134 kick-sets at Moore's Ferry, Licking River, Kentucky.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	2.101	1.122	0.957	0.456
Proportion of total variance	0.420	0.224	0.191	0.093
Eigenvectors:				
Current Velocity	0.5841	-0.1745	0.1807	0.2380
Substrate Size	0.0603	0.7842	-0.5097	0.3075
Substrate Variability	0.0355	0.5473	0.8282	0.0298
Depth	0.5897	-0.1259	-0.0455	0.4726
Percent Coverage, *Potamogeton*	-0.5533	-0.1979	0.1399	0.7903

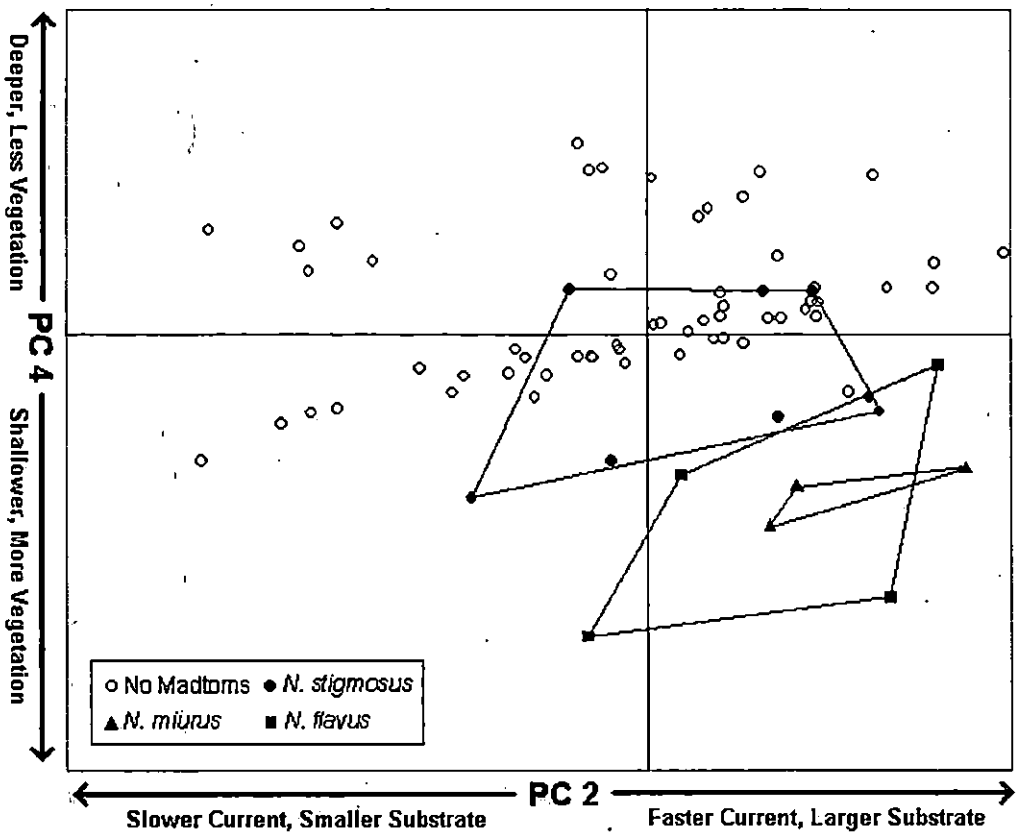


Figure 15. Scatterplot of 134 kick-sets conducted at Moore's Ferry along multivariate axes generated by PCA.

Noturus stigmosus also occupied more varied habitat types than either *N. flavus* or *N. miurus*. Principal components 2 and 4 were chosen, together accounting for over 31% of the total variance. Although other combinations of axes accounted for more variance, clustering of madtoms along these axes failed to reveal meaningful patterns of habitat partitioning. Components accounting for less variance should not be assumed *a priori* to be less important than those accounting for more (Wiley, 1981). In fact, percent variance accounted for by a component does not strongly predict its ecological utility, and a given component must be assessed in terms of the information it provides (Matthews, 1985).

Results of multivariate analysis were consistent with results of the univariate analysis. The cluster of *N. stigmosus* overlapped that of *N. flavus* somewhat, but the *N. stigmosus* was encountered in areas with deeper water and less vegetation than either of the other two madtoms at Moore's Ferry. Habitat preferences of *N. miurus* almost completely overlap those of *N. flavus*.

Diet

Gut contents of 83 *Noturus stigmosus* were examined; only two were empty or contained unidentifiable digested material. The overall diversity of the diet was high; 27 families of invertebrates were found in the stomachs of *N. stigmosus*. Diversity was also high within major orders; Diptera was represented by six families, and Coleoptera, Ephemeroptera, and Trichoptera by five (Table 7).

The vast majority of prey items were insects (24 families), which was dominated by a few major taxa. Dipteran, trichopteran, and ephemeropteran immatures composed 97% of the overall diet, accounting for 55.8%, 18.0%, and 23.2% of total prey organisms, respectively. Chironomid larvae accounted for 86.2% of the dipterans eaten, and simuliid larvae and pupae comprised another 11.6%. Emphemerellidae accounted for more of the ephemeropterans consumed (55.6%), but Heptageneiidae (43.1%) most likely dominated in biomass due to their much larger body size. Trichopteran contributors were dominated by Hydropsychidae (41.3%), Hydroptilidae (39.6%), and Limnephilidae (11.0%). One stomach contained a parasitic acanthocephalin, and another contained three nematodes, which were possibly parasitic as well. Numerous stomachs contained plant material (e.g. seeds, leaves, bark, and filamentous algae), as well as sand and small pebbles. These were probably parts of trichopteran larval or pupal cases or were debris accidentally ingested with food items during suction feeding (Burr and Stoeckel, 1999; Matthews, 1998).

Table 7. Stomach contents of 81 *Noturus stigmosus* from Moore's Ferry, Licking River, Kentucky, based on enumeration of prey items. Numbers to the left are percentages of diet derived from each food organism, those in parentheses are percent of stomachs in which food organisms occurred.

Food Organism	Size Class ≤ 50 mm SL (N = 33)	53-61 mm SL (N = 33)	≥ 64 mm SL (N = 15)
Malacostraca			
Decapoda			
Cambaridae	-----	-----	0.3 (6.7)
Insecta			
Coleoptera			
Carabidae	-----	0.1(3.0)	0.6 (13.3)
Elmidae	0.1 (6.1)	0.2 (3.0)	0.3 (6.7)
Gyrinidae	0.8 (12.1)	2.0 (30.3)	3.9 (26.7)
Hydrophilidae	0.1 (6.1)	1.0 (12.1)	-----
Staphlynidae	0.06 (3.0)	0.1 (3.0)	-----
Diptera			
Ceratopogonidae	0.6 (18.1)	1.5 (24.2)	1.5 (6.7)
Chironomidae	59.9 (87.9)	37.0 (84.8)	25.7 (66.7)
Empididae	-----	-----	0.6 (6.7)
Muscidae	0.06 (3.0)	-----	0.3 (6.7)
Simuliidae	5.8 (48.5)	5.2 (42.4)	13.3 (60.0)
Tipulidae	0.06 (3.0)	-----	0.3 (6.7)
Ephemeroptera			
Ameletidae	0.1 (6.1)	-----	-----
Baetidae	-----	0.1 (3.0)	-----
Ephemerellidae	11.2 (66.7)	10.2 (36.4)	3.9 (33.3)
Heptageneidae	2.5 (54.5)	11.7 (69.7)	20.8 (33.3)
Leptophlebiidae	0.06 (3.0)	0.2 (3.0)	-----
Hemiptera			
Notonectidae	-----	0.1 (3.0)	-----
Lepidoptera			
Pyralidae	0.1 (6.1)	0.1 (3.0)	-----

Table 6. (Cont.)

Food Organism	Size Class ≤ 50 mm SL (N = 33)	53-61 mm SL (N = 33)	≥ 64 mm SL (N = 15)
Odonata			
Calopterygidae	-----	0.1 (3.0)	0.3 (6.7)
Coenagrionidae	-----	0.1 (3.0)	-----
Plecoptera			
Pteronarcyidae	0.4 (9.1)	0.1 (3.0)	0.3 (6.7)
Trichoptera			
Brachycentridae	0.3 (9.1)	2.8 (30.3)	1.2 (20.0)
Hydropsychidae	5.3 (69.7)	14.5 (60.6)	15.7 (53.3)
Hydroptilidae	9.8 (10.7)	9.6 (39.4)	1.2 (20.0)
Limnephilidae	0.9 (21.2)	2.3 (30.3)	9.6 (40.0)
Unidentified Pupae	0.6 (21.2)	1.0 (18.1)	0.3 (6.7)
Nematoda	0.2 (3.0)	-----	-----

A comparison of gut fullness in nocturnally and diurnally-collected *N. stigmosus* is shown in Figure 16. Individuals collected during the day generally had empty or partially full fore- and midguts, but the hindgut was typically full. Individuals collected at night typically had full foreguts and partially full or empty mid- and hindguts, although hindgut fullness was greater that expected.

The diet of *N. stigmosus* differed between summer and autumn (Figure 17). Generally, the proportions of Coleoptera, Ephemeroptera, and Trichoptera were higher in summer than in fall, and Diptera, a major component in both seasons, became the dominant prey taxon in fall. Hydropsychidae and Heptagenidae made up large portions of the diet in summer, but the percentages were halved in autumn. Coleoptera were significant contributors to the summer diet, but were largely absent in fall. Chironomidae dominated the diet in both seasons, but was twice as abundant in the diet in fall relative to summer. Ephemerellidae and Hydroptilidae also became important components of the diet in autumn, however this shift in dominance towards smaller prey may be due in part to a large percentage of age class 0 madtoms being sampled at this time.

Larger individuals tended to eat larger prey items. The relatively small-sized members of Chironomidae, Emphemerellidae, and Hydroptilidae in the diet decreased with increasing size of the madtom, while the relatively large members of Simuliidae, Heptaganeidae, and Limnephilidae all increased with madtom size class (Table 7). The largest prey item consumed, a cambarid crayfish (*Orconectes cristivarius*), was found in the stomach of an individual 73 mm SL.

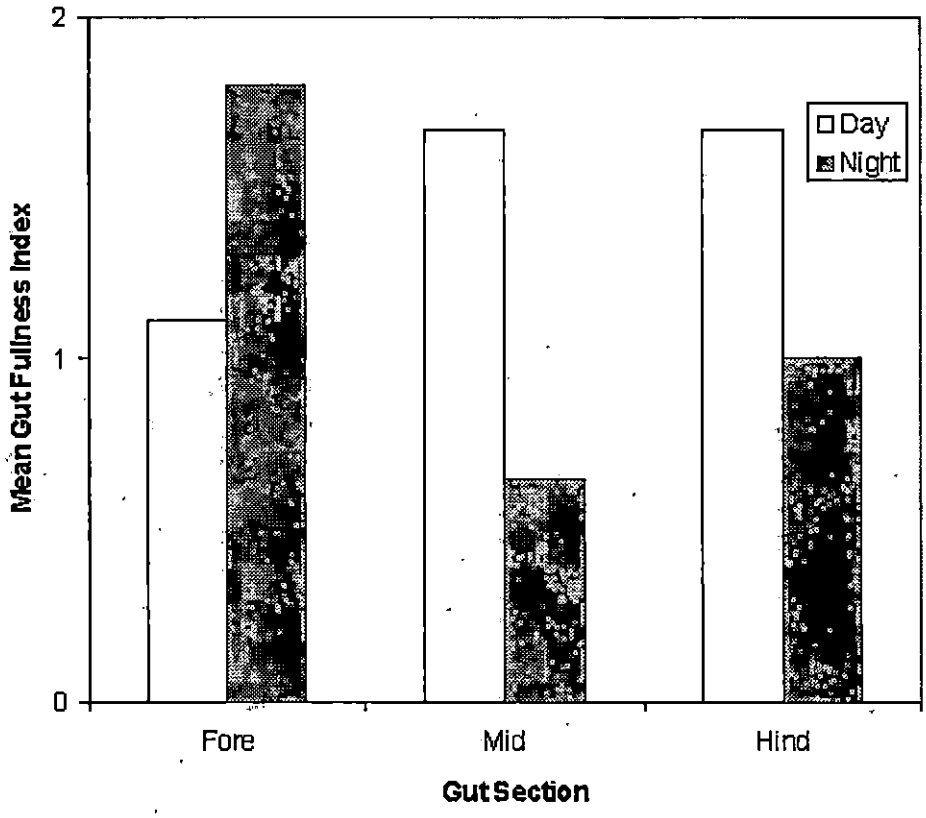
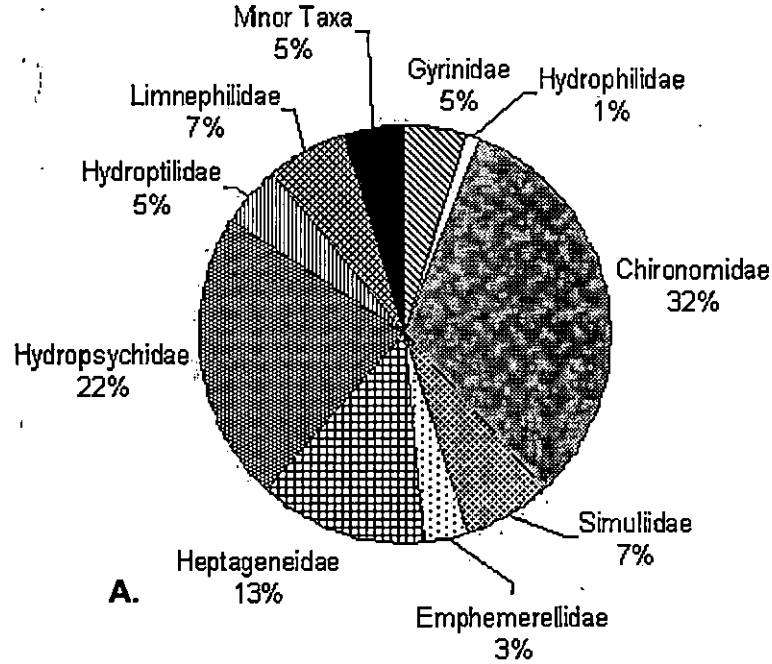


Figure 16. Gut fullness as determined from visual estimation for madtoms collected 20 (day, n = 9) and 22 (night, n = 20) September 2002.



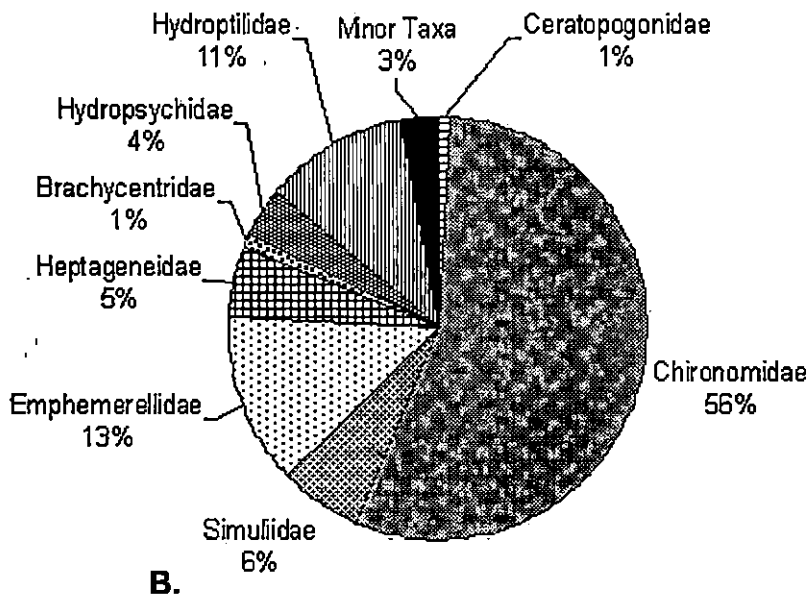


Figure 17. Percent composition of food items of *N. stigmosus* at Moore's Ferry in (A) summer (n = 40) and (B) fall (n = 41). Minor taxa were families that accounted for < 1% of the diet numerically.

DISCUSSION

Demography

The population of *N. stigmosus* at Moore's Ferry was dominated by YOY in 2002, yet this age class was practically nonexistent in collections made in 2001. Of 188 individuals collected in 2002, 151 (80.3%) were YOY, 21 (11.2%) were age 1 fish, and 16 (8.5%) were age 2 or older. A population skewed toward younger age classes indicates normal recruitment (Burr et al, 1989; Starnes and Starnes, 1985), but this was not always seen at this site.

Historically, the Moore's Ferry population of *N. stigmosus* has been extremely volatile (pers. comm., David Eisenhour). The period between June 2001 and October 2002 was no different; catch per unit effort and population density estimates increased by a factor of ten over the duration of the study. During this time period, a major difference between collecting seasons was in precipitation and resulting water releases from the Cave Run Lake impoundment. The spring and early summer of 2001 were extremely wet in eastern Kentucky. Consequently, the water releases from Cave Run Dam were well above normal, resulting in flooding the field site to levels greater than the "two standard deviation rule" used to classify floods large enough to impact fish populations (Matthews, 1998). In addition, water released from Cave Run Lake is usually hypolimnetic, much colder (at least 5 - 10°C) than ambient stream temperature (personal observation).

This flood event could have adversely impacted the Moore's Ferry population of *N. stigmosus* in several ways, including removal of individuals downstream,

destruction of habitat, and disruption of spawning (Matthews, 1998). Inspection of the site after high flows receded showed that major areas of the riffle had been restructured (e.g. gravel bars moved), but most of the potential habitat remained. It is hard to say if washing of individuals downstream was a major factor affecting population numbers, considering that madtoms could take shelter under large slab rocks or bury in the substrate. Reproduction could have been interrupted by the flood in a variety of ways, including destruction of nest sites and eggs, movement of bed load, and cooling the temperature of the stream below the critical spawning temperature (Matthews, 1998), approximately 20°C for *Noturus* (Burr and Stoeckel, 1999).

The first two scenarios are difficult to quantify and document, the third is not. Spikes in gage height in early July through August 2001 (Figure 18) correspond to sharp drops in water temperature when ambient stream temperature was approaching 20°C (Figure 19). These prolonged floods twice lowered the water temperature to 15 - 18°C, preventing the ambient stream temperature from reaching a sustained 20°C until it was too late in the year for spawning. Thus, the Moore's Ferry population potentially missed recruitment from an entire year.

There was less precipitation in the summer of 2002, and flow regimes were more typical than in 2001. Catch per unit effort and population density estimates were more than ten times higher in 2002 than the previous year. A nest was found, and YOY, which were virtually absent in 2001, dominated the collections from mid-

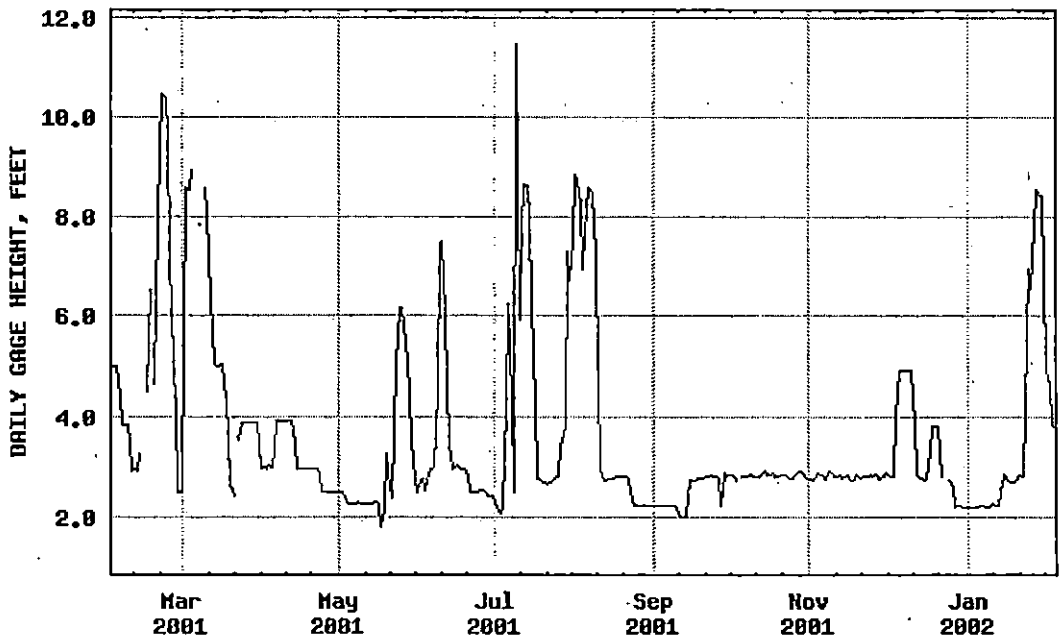


Figure 18. Gage height data from the Licking River at Farmers, Kentucky, approximately 10 km upstream of Moore's Ferry, 1 February 2001 - 4 February 2002.

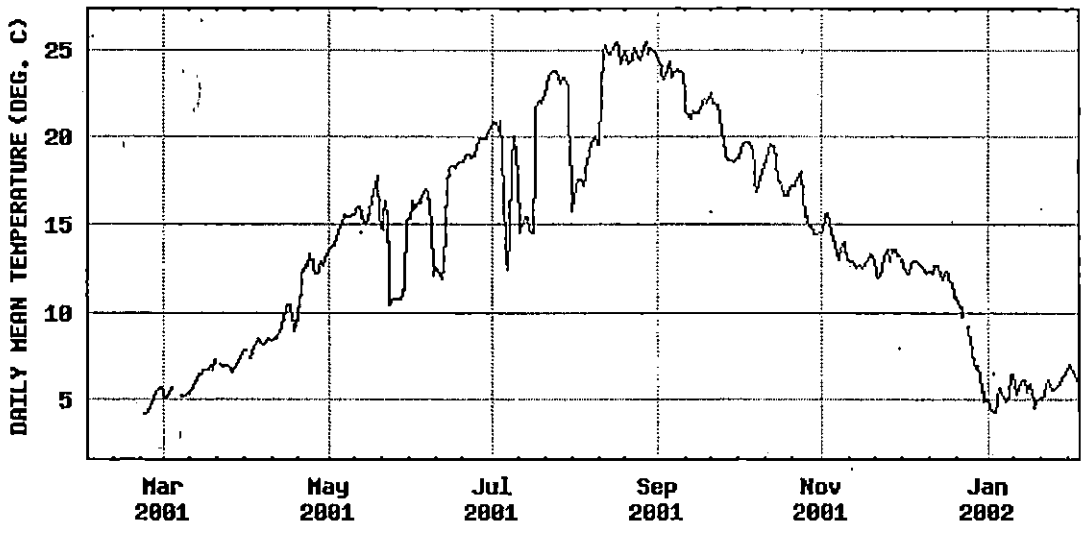


Figure 19. Instream temperature of the Licking River at Farmers, Kentucky, approximately 10 km upstream of Moore's Ferry, 1 February 2001 - 4 February 2002.

July through October. The response of the population to the flood could suggest that it could be regulated by density independent factors, such as water temperature, especially in light of the apparent surplus of available madtom habitat (Table 4).

It has been suggested that cold hypolimnetic releases from reservoirs limit reproduction and growth in *N. gilberti* (Simonsen and Neves, 1992), and Taylor (1969) reports that madtom populations can disappear within one to two years below newly-constructed dams. Successive year-class failures could result in local extirpations or extreme fluctuations in population levels for madtoms (Simonsen and Neves, 1992), similar to what was seen at Moore's Ferry.

The lifespan of *N. stigmosus* at Moore's Ferry compares well to Burr's estimate of two to three years for the species (Burr and Stoeckel, 1999), but is somewhat shorter than that of other medium-sized madtoms living in stream environments. At the site, individuals typically live two years, with a few surviving until their third year. Although it would be difficult to separate an age class 3 from the length-frequency analysis of the population, there are likely fish older than 2 years included in age class 2+. Sizes range between 65 and 85 mm SL within this group, at a time in life when growth is at is lowest rate (Figure 6).

Once juveniles leave the nest, high mortality is expected due to the high number of potential predators. This mortality decreases as the fish get larger; rapidly-growing YOY are soon too large for invertebrate predators (e.g., crayfish, large odonate nymphs) to handle, piscivorous predators large enough to consume

madtoms cannot invade the shallow water of the riffle (Schlosser, 1987), and the venomous spines of larger madtoms presumably become more of a deterrent.

Growth

The growth regression of *N. stigmosus* is similar to published accounts of other similarly sized madtoms (e.g., *N. exilis*, Mayden and Burr, 1981). Trautman (1981) reported that YOY *N. stigmosus* reach 25 - 58 mm TL by four to five months, and MacInnis (1998) recorded similar data in Lake St. Clair, Ontario. Young-of-the-year in Canada spawned in July reached at least 30 mm TL by October. Growth rates for YOY at Moore's Ferry are similar, but YOY attain larger sizes than those from Canada (37 – 48 mm SL) (Figures 4 and 5), likely due to the longer growing season at lower latitudes.

The largest adult *N. stigmosus* Trautman (1981) captured from Lake Erie measured 130 mm TL, and MacInnis (1998) reports a maximum size of 129.8 mm TL from Lake St. Clair. Maximum size at Moore's Ferry was less than either Lake St. Clair or Lake Erie at 87 mm SL (approximately 100 mm TL), but madtoms in northern lakes tend to live longer and grow larger than the individuals of the same species in southern stream environments (Burr and Stoeckel, 1999).

The relationship between length and weight shows that females are heavier at smaller sizes, but males overtake them at about 50 mm SL, and males reach greater maximum adjusted weights (Figure 7). Adjusted weight essentially reflects muscle

and skeletal mass; the difference between the sexes suggests that male *N. stigmosus* put more resources into somatic growth than do females.

In many madtom species, males generally have greater adjusted weights than females (e.g., *N. flavipinnis* (Dinkins and Shute, 1996) and *N. flavater* (Burr and Mayden, 1984)). It is suggested that this is due to sexual selection for more massive males, as they procure, prepare, and defend nest sites. Larger males would be selected for because they are better nest defenders and would be less likely to leave the nest site if disturbed (Burr and Mayden, 1984). Males also feed little, if at all, while guarding the nest (Burr and Stoeckel, 1999); larger males would be able to resist starvation better than smaller males, a pattern seen in other fishes with male nest guarding (Matthews, 1998).

Females, on the other hand, divert more of their resources into oocyte production; they do not need to defend territories and oocytes are physiologically expensive to produce, requiring more energy from the diet for gonad development (Dinkins and Shute, 1996).

An alternative hypothesis has been proposed for several species, including *N. miurus* (Burr and Mayden, 1982a), *N. eleutherus* (Starnes and Starnes, 1985), and *N. exilis* (Mayden and Burr, 1981), stating that males attain larger sizes because they live longer. Although not subject to statistical tests, larger size classes appeared to be evenly divided between males and females of *N. stigmosus* at Moore's Ferry, suggesting that survivorship was equivalent between the sexes in older age classes.

Reproductive Biology

The spawning season of *N. stigmosus* at Moore's Ferry potentially runs from mid-June to late July, depending on ambient water temperature. Both sexes show high GSI values and development of secondary sexual characteristics from mid June to mid July, followed by a rapid decline in late July to early August (Figures 9 and 11). The discovery of a nest, apparently spawned in late June, and two reproductive males apparently guarding prepared nest sites in mid-late June support the identification of an early to mid summer spawning season.

Noturus stigmosus reaches sexual maturity in the second summer of life, at approximately 60 mm SL. Males' GSI values reach an inflection point and begin to rapidly increase when they reach 60 - 65 mm SL (Figure 8); female GSIs rise rapidly as they reach about the same size, at 58 - 60 mm SL (Figure 10). Individuals smaller than these size ranges never displayed well-developed secondary sexual characteristics. However, some evidence for early maturation of females was found; females approximately 55 mm SL had GSI values much higher than what was expected for a fish of that size. Variation in age at reproductive maturity within populations has been seen in other fish species as a response to predation (Belk, 1995), the existence of multiple reproductive strategies (Matthews, 1998), and ambient environmental conditions (Bayliss et al., 1993).

In *N. stigmosus*, environmental factors are likely to influence age at reproductive maturity. Early maturation in females has been documented in other species of madtoms, including *N. flavipinnis* (Dinkins and Shute, 1996) and *N.*

flavater (Burr and Mayden, 1984). This is thought to occur only under optimal conditions of warm winter water and abundant food, based on laboratory maintenance of madtoms (Dinkins and Shute, 1996). An unseasonably warm summer with abundant insects could have allowed females at Moore's Ferry to grow quickly, potentially reaching sexual maturity near the end of the spawning season, when they were 13 to 14 months old.

Clutch size for *N. stigmosus* must be at least 40 eggs in light of the nest contents found, and is estimated at 70 - 100 eggs based on mean fecundity (76.5 eggs/female). This estimate falls within the range Taylor (1969) reported for females from Michigan, and closely follows the relationship between mean fecundity and body size Burr and Stoeckel (1999) presented for ictalurids. MacInnis (1998) reported clutch sizes of 32 -140 eggs for Lake St. Clair in Ontario, but believed the largest egg nest is the result of two females' reproductive output. However, this could actually be from one female, given the large size of *N. stigmosus* from northern lakes. The estimated clutch size for the Moore's Ferry population and others published accounts fall within the reproductive potential of one female of their respective populations, so *N. stigmosus* is likely iteroparous.

Nesting habitat at Moore's Ferry is comparable to Taylor's (1969) account for the species in the Detroit River in Michigan. In both cases, nests were found in riffle areas in moderate currents and depths. Clutches were found in depressions below slab rocks, and evidence for nest preparation was seen. However, no guardian adults were found at Moore's Ferry, but they were present in the Detroit River. This is not

surprising, as the clutch found at Moore's Ferry contained metalarvae estimated at two weeks of age, about the time when juvenile madtoms begin to disperse from the nest (Burr and Stoeckel, 1999). Taylor (1969) also reported finding *N. stigmosus* utilizing human refuse (e.g., broken crockery) as nesting substrate, but this was not observed at Moore's Ferry.

Although the accounts compare well, more data are needed to compare nesting habits of *N. stigmosus* throughout its range. This study presents only the second record of a nest from a stream environment, and now that the species has been extirpated from the type locality, it represents the only documentation of nesting from an extant stream population.

Habitat

Noturus stigmosus probably spends the daylight hours under rocks or buried in substrate interstices. Often, very few *N. stigmosus* were encountered during snorkeling surveys of likely habitat, but when these same areas were seined, with vigorous disturbance of the substrate, up to eight individuals were captured in a single seine haul. Burying in substrates has been documented in two other madtom species, *N. eleutherus* (Starnes and Starnes, 1985) and *N. exilis* (Stegman and Minckley, 1959).

There is some evidence of microhabitat partitioning between age classes of *N. stigmosus* at Moore's Ferry. Juveniles occupied areas with habitat parameters within the range of those occupied by adults, but multivariate analysis revealed a tight

grouping of juvenile *N. stigmosus* associated with areas of slower current, shallower water, and more vegetative cover than adults (Figure 12). This may reflect juvenile habitat preferences and physiological limitations rather than a mechanism to reduce intraspecific competition. Juvenile fishes often occupy areas with lower flow to accommodate reduced swimming ability, and occupy shallower areas and those with cover to avoid predators (Schlosser, 1987).

Habitat preferences of *N. stigmosus* at Moore's Ferry compare well with those recorded from streams elsewhere in its range. Taylor (1969) described collecting the species in large streams and small rivers in Michigan in areas with moderate current over mixed substrates of sand, gravel, and cobble with some vegetation. Holm and Mandrak (2001) described habitats in the Detroit and Thames Rivers in Ontario as being 1 - 3 m deep with moderate current, over substrates of sand, gravel, rubble, and occasional submerged aquatic vegetation. Cooper (1983) reported a trend of preference for greater current velocities in more northern populations of *N. stigmosus*. However, comparisons are difficult to make without quantitative data. This study considers current velocities of ~0.5 m/sec "moderate" relative to those recorded at Moore's Ferry, and without actual numbers, it is difficult to determine what current velocities other authors consider "moderate", "fast", or "swift" when describing habitat, but the data recorded in this study indicate that the Moore's Ferry population falls within this pattern.

Relative to the available habitat at Moore's Ferry, madtoms occupied areas with greater current velocities and larger substrate sizes (Figure 15). Of the three

species, *N. stigmosus* occupied a wider range of habitat variables than either *N. flavus* or *N. miurus* (Figure 14). *Noturus stigmosus*, while having a wide distribution, is generally found only in locally abundant populations. Several collecting attempts were made in 2001 in other areas of the Licking River where the species was expected to be, and had been found; none were successful. It appears that *N. stigmosus* has very specific habitat requirements, and the persistence of the Moore's Ferry population suggests the site possesses features required by the fish. In fact, the majority of the available habitat at the site is suitable for *N. stigmosus* (Table 4), and much of the unoccupied habitat falls within the range of the species' preferences (Figure 15). Moore's Ferry may be an optimal site for the species; more research needs to be conducted to identify the factors that allow for persistence of the species despite the irregular flow regime.

Significant statistical differences and separation along multivariate axes were seen for the habitat preferences of *N. stigmosus* and *N. flavus* at Moore's Ferry. *Noturus stigmosus* was found in areas with faster current, deeper water, and less vegetation than *N. flavus*. It was expected that *N. flavus* would occupy deeper areas because of its larger size (Schlosser, 1987), but occupation of shallower areas could be an artifact of the large proportion of juveniles in the sample.

Closely related species may partition resources such as food and habitat (Hansen et al., 1986), resulting from interference competition due to similar resource utilization. Competition is more intense between cogeners than between more distantly related species (Matthews, 1998), and benthic fishes can only partition

habitat horizontally, further intensifying competition (Finger, 1982). Competition between the madtom species at Moore's Ferry was therefore expected to be intense, resulting in distinct habitat partitioning between *N. stigmosus, N. flavus,* and *N. miurus.*

Resource partitioning studies intend to analyze the limits of competition (Schoener, 1974), with resource segregation between species demonstrating its existence. However, the data must be interpreted carefully; there must be more evidence of competition than use of the same resources. In field situations, overlap of resource utilization can be seen at both ends of a continuum of actual competition. At one extreme, resources are superabundant and many species use the same resource (e.g., many fishes feeding on emerging mayflies), but little, if any, ecologically meaningful competition occurs. At the other end, resources are extremely rare, and several species may compete intensely for them. In practice, most systems lie in between these extremes, so available resources must be compared to utilization to describe competition in a meaningful way (Matthews, 1998).

Although microhabitat partitioning may be seen between the three madtom species at Moore's Ferry, little evidence for competition was found. Over half of the potential habitat surveyed was suitable for multiple madtom species (Table 4). However, little actual utilization of the available habitat was seen. All species occupied less than 30% of the available habitat for the species, and only 5% of the habitat suitable for multiple species was occupied by more than one madtom (only two seine hauls out of 309 yielded more than one species). This suggests at Moore's

Ferry microhabitat partitioning is more likely a result of madtom habitat preferences rather than interspecific competition.

Competition was found not to be limiting populations of *N. placidus* in the Neosho River and its tributaries. Instead, density independent factors and anthropogenic impacts on water quality and habitat from stream impoundment were suggested to be responsible for low population densities of the fish (Wildhaber et al, 1999). Similarly, *N. stigmosus* populations appear to be controlled by the success of recruitment, which may be affected by ambient stream temperature. Temperature can be greatly influenced by water releases below reservoirs, and this can in turn suppress spawning (Matthews, 1998). Most published accounts cite a narrow range of spawning temperatures for madtoms from 20 - 25°C (Burr and Stoeckel, 1999), so cold water releases from Cave Run Lake could be limiting not only *N. stigmosus* at Moore's Ferry, but also *N. miurus* and *N. flavus*.

Diet

Noturus stigmosus is a gape-limited, nocturnal, opportunistic insectivore, based on the relationship of prey size to body size, timing of feeding, and composition and variety of the overall diet. *Noturus stigmosus* consumed a wide variety of prey taxa at Moore's Ferry. Twenty-seven families of invertebrates were identified from the guts of *N. stigmosus* (Table 7). Over 98% of the total diet was composed of insects, dominated by Diptera, Ephemeroptera, and Trichoptera. One or two families usually dominated, but diversity was high within prey orders; Diptera

was represented by six families, and Coleoptera, Ephemeroptera, and Trichoptera by five each.

The gut contents of benthic riffle fishes, including *N. miurus* (Burr and Mayden, 1982) and *N. munitus* (Miller, 1984) closely reflect the relative occurance of prey taxa available in the environment. The dominant taxa in the diet of *N. stigmosus* are nocturnally active (Merrit and Cummins, 1984). Chironomidae and Simuliidae drift at night (Waters, 1972), and *N. stigmosus* could take individuals as they settled to the substrate. Feeding on drifting organisms has been documented in other madtoms, including *N. exilis* (Mayden and Burr, 1981) and *N. eleutherus* (Starnes and Starnes, 1985), but may be restricted to riffle-dwelling madtoms (Dinkins and Shute, 1996).

Seasonal prey availability is reflected in the diet of *N. stigmosus*. Trichoptera and Emphemeroptera were important prey organisms in the summer diet during their peak abundance (Merrit and Cummins, 1984), but made up much less of the diet in fall (Figure 17). Diptera, while important components of the diet in both seasons, double in abundance in the fall diet, making up 63% of the total in this season. This reflects the relatively ubiquitous presence of the order at the study site throughout the year.

Other prey taxa give clues to where *N. stigmosus* forages at Moore's Ferry. Many of the ephemeropteran nymphs consumed were clingers (Merrit and Cummins, 1984), and Brachycentridae larvae exclusively were found attached to *Potamogeton*;

these taxa were likely taken by *N. stigmosus* foraging under rocks and among the submerged vegetation.

The diet of *N. stigmosus* differs considerably between the population at Moore's Ferry and those in Canada, as reported by Holm and Mandrak (2001). Stomachs from specimens collected in the Detroit River and the St. Clair River contained mostly insects, although some small *Notropis volucellus* and *Neogobius melanostomus* were found. Diptera, Ephemereoptera, and Trichoptera dominated, but various small Crustacea, Lepidoptera, Plecoptera, and Nematoda also were present in significant numbers. At Moore's Ferry, Diptera dominate the diet, with significant numbers of Ephemeroptera and Trichoptera, however, Teleostei and Crustacea are largely absent.

Mouth size and body size are tightly coupled in fishes (Matthews, 1998), so generally, small fishes must consume small prey. Gutowski and Stauffer, Jr. (1993) indicate gape-limited predation in *N. insignis* is based on smaller individuals consuming smaller prey. This relationship is also seen in *N. stigmosus*; families of smaller insects within major orders (e.g., Chironomidae, Ephemerellidae, and Hydroptilidae) dominated the diet of smaller size classes, while larger taxa (e.g, Simuliidae, Heptageneiidae, and Hydropsychidae) were most important in the largest size class (Table 7).

Although some mode of selective feeding is employed by *N. stigmosus*, it is unclear if this is optimal foraging. Generally, fishes following an optimal foraging strategy consume larger prey than what is encountered on average in the environment.

Larger fish tend to follow the optimal model closer than smaller fish, as their searching and prey handling abilities are more efficient than those of smaller fish (Mittlebach, 1981). Larger fish ate larger prey items, but small chironomids were still important in their diets. There was also an upper size boundary for prey, as large immature insects (e.g., *Pteronarcys* stoneflies and *Hagenius* dragonflies) were abundant at Moore's Ferry (personal observation), but were largely absent in the diet (Table 7). The diet of *N. stigmosus* may reflect the tendency for fishes to eat prey from a wide range of sizes, as long as the prey item can be handled efficiently (Matthews, 1998).

The results of the comparison of diel and nocturnal gut contents agree with the results of Burr and Mayden's (1981) study of periodicity of stomach fullness and digestion index of stomach contents for *N. exilis*. In this study, they report that stomach fullness showed a bimodal peak at night, and that gut contents were less digested than during the day. Stomachs from day-collected *N. stigmosus* occasionally contained prey items, suggested limited daytime feeding, a phenomenon reported for both *N. gilberti* (Simonsen and Neves, 1992) and *N. munitus* (Gutowski and Stauffer, Jr., 1993). Hindguts from individuals collected at night did show a greater volume of digested material than expected, but this was probably prey consumed the night before that had not been voided yet. Starnes and Starnes (1985) found that the mean gut passage time for a meal was 24 hours in *N. eleutherus*, and that material in the hindgut from the previous nights feeding is passed early in the evening as foraging begins.

Summary

This study documented the habitat preferences, diet, demography, reproductive cycle, nesting habits, intraspecific competition, and resource partitioning of *N. stigmosus* at Moore's Ferry. It also examined interspecific competition and habitat partitioning between the three syntopic madtom species at the site.

Noturus stigmosus commonly were collected within the main body of the riffle at Moore's Ferry, and appeared to avoid pool habitats above the riffle, and raceway habitats downstream. Within the riffle, the species preferred areas of moderate current and depth over mixed substrates of sand, gravel, and cobble. Juveniles were associated with shallower areas with slower currents at the head of riffles, and areas with more vegetation than adults. *Noturus stigmosus* is nocturnal, apparently spending the daylight hours buried in substrate interstices.

Noturus stigmosus consumed a wide variety of prey, all benthic invertebrates. Twenty-four of twenty-seven families identified from gut contents were insects, dominated by Diptera, Ephemeroptera, and Trichoptera. Overall diet composition followed diel and seasonal prey abundances, and size of prey consumed was related to body size. Peaks in gut fullness indicate *N. stigmosus* is a nocturnal forager.

The species lives at least two to three years at Moore's Ferry, with three distinct age classes. In 2002, the population was skewed towards younger age classes, indicating good recruitment this year. However, recruitment was poor in 2001, due to the interruption of spawning by a major, sustained flooding event in late June through mid July.

Noturus stigmosus reaches sexual maturity during the second summer of life, when individuals attain approximately 60 mm SL, although some evidence of early maturation in females was found. Males and females both come into reproductive condition in early summer and display marked secondary sexual characteristics typical of madtoms. The single nest discovered over the course of the study was found during this period of peak reproductive potential. Egg clutches are laid in cavities below slab rocks within the main body of the riffle. Clutch sizes are estimated at 70 – 100 eggs, based on the number of larvae found and gross examination of ovaries of reproductively mature females in spawning condition.

This study was not able to document the early development of *N. stigmosus*, due to the failure of attempts to rear madtom larvae. However, development is likely similar to that of other madtoms; the *N. stigmosus* larvae found were at the same stage of development as other species at the same size reared in a laboratory setting. Overall growth was rapid for YOY, but decreased rapidly as individuals reached approximately one year of age.

Evidence for resource partitioning was seen between *N. stigmosus* YOY and adults, as well as between syntopic madtoms at Moore's Ferry. Adult and juvenile habitat preferences were continuous, but juveniles occupied a narrow range within those of adults, likely based on predator avoidance and juvenile swimming ability. Segregation was also seen between madtom species; *N. stigmosus* was found in deeper areas with faster currents and less vegetation than *N. flavus*. Multivariate analysis supported this, and showed separation between *N. stigmosus* and *N. miurus*.

Overlap existed between madtom species, especially between *N. miurus* and *N. flavus*. Little evidence was found for interspecific competition for habitat, and the madtom assemblage may be controlled by density-independant factors instead.

Implications for Conservation

Flow regimes in a given area can have much influence on the evolution of life history strategies of fishes (Matthews, 1998), so anthropogenic changes in flow (e.g., from dam releases) could have significant impacts on fish populations. *Noturus stigmosus*, with small clutch sizes, large egg diameters, high parental care, and small body size, falls within Winemiller and Rose's (1992) definition of an "equilibrium" life history strategy. These fishes maximize juvenile survivorship by investing resources in propagule size and parental care (Pianka, 1999). However, this life history strategy has no selective advantage in environments driven by seasonal or density-independent selection (Winemiller and Rose, 1992). Single spawning bouts typical of equilibrium species could easily result in the loss of an individual's entire reproductive effort for a season if conditions are poor when it spawns (Matthews, 1998).

This likely happened to the Moore's Ferry population of *N. stigmosus* in the summer of 2001. Cold water releases of sufficient magnitude and duration to suppress reproduction in this year resulted in poor recruitment and low population densities when compared with 2002, a year with a more typical flow regime. While the population could rebound from a poor reproductive effort one year, suppressed

reproduction over two or more years could extirpate the species from Moore's Ferry as individuals here typically live only 2 - 3 years. The importance of water quality (Holm and Mandrak, 2001) and habitat preservation (Goodchild, 1993) have been identified as critical factors in the conservation of *N. stigmosus*, but the timing, magnitude, and duration of water releases from impoundments must be considered in future management plans for the species. Releases from Cave Run Dam reflecting a flow regime more typical of the Licking River before impoundment would be ideal, similar to the program being implemented along the Green River in Kentucky. If this is not possible, at least avoiding prolonged cold water flows during the spawning season in June and July would ensure some reproductive success for *N. stigmosus* at Moore's Ferry.

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